CANINE AND FELINE PROTEINS, NUCLEIC ACID MOLECULES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Serial No. 60/223,016, which was filed August 4, 2000, entitled "CANINE AND FELINE PROTEINS, NUCLEIC ACID MOLECULES AND USES THEREOF".

FIELD OF THE INVENTION

The present invention relates to canine and feline proteins. In particular, the present invention relates to feline interleukin-18 (IL-18), feline caspase-1 (casp-1), feline interleukin-12 (IL-12) single chain, and canine interleukin-12 (IL-12) single chain proteins and includes nucleic acid molecules encoding such proteins, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to evaluate and regulate an immune response in an animal.

BACKGROUND OF THE INVENTION

Regulation of immune and inflammatory responses in animals is important in disease management. Immune responses can be regulated by modifying the activity of immunoregulatory molecules and immune cells. Examples of such immunoregulatory molecules include IL-18, caspase-1 and IL-12. These molecules have been found to play a role in the treatment of several disorders including allergy, cancer, and pathogenic infection.

Monocytes and macrophages represent the first line of defense against disease. Various diseases and infections activate transcriptional and posttranslational events in monocytes and macrophages, which lead to the production of cytokines such as IL-18 and IL-12. These cytokines in turn activate responses in T and B cells helping to eliminate pathogens and/or disease in a animal. Both IL-18 and IL-12 augment cellular immunity by stimulating T cells to produce interferon gamma (IFN-γ) which inhibits the production of IgE formation without compromising B cell proliferation. IL-18, formerly referred to as interferon gamma inducing factor (IGIF), stimulates T cells to produce IFN-γ and has been isolated from humans, dogs, and mice. A cDNA encoding human IL-18 was isolated and used to express recombinant human IL-18 by Ushio et al., 1996, J. Immunol.156, 4274-4279, GenBank accession number D49950. Feline IL-18 cDNA has a 85.8% homology to human IL-18 cDNA and feline IL-18 protein has a 81.7% homology to human IL-18 protein. A cDNA encoding canine IL-18 was isolated and used to express recombinant canine IL-18 by Okano et al., 1999, J. Interferon Cytokine Res. 19, 27-32, GenBank accession number Y11133. Feline IL-18 cDNA has a 90.7% homology to canine IL-18 cDNA and feline IL-18 protein has a 88.5% homology to canine IL-18 protein. A cDNA encoding murine IL-18 was isolated and used to express recombinant murine IL-18 by Okamura et al., 1995, Nature 378, 88-91, GenBank accession number D49949. Feline IL-18 cDNA has a 73.8% homology to murine IL-18 cDNA and feline IL-18 protein has a 70% homology to murine IL-18 protein. A cDNA encoding rat IL-18 was isolated by Culhane, et al. Mol. Psych. 3, 362-366 (1998), GenBank accession number AJ222813. Feline IL-18 cDNA has a 73.4% homology with rat IL-18 cDNA, and feline IL-18 protein has a 70.7% homology with rat IL-18 protein.

A cDNA encoding equine IL-18 was isolated by Nicolson, et al. (unpublished, direct submission to GenBank, accession number Y11131). Feline IL-18 cDNA has a 92% homology to equine IL-18 cDNA and feline IL-18 protein has a 89% homology to equine IL-18 protein. A cDNA encoding pig IL-18 was isolated by Penha-Goncalves, et al. (unpublished, direct submission to GenBank, accession number Y11132. Feline IL-18 cDNA has a 90.2% homology to pig IL-18 cDNA and feline IL-18 protein has a 85.9% homology to pig IL-18 protein. Expression of active IL-18 is controlled by caspase-1(IL-1β converting enzyme). That is, IL-18 lacks a signal peptide so the precursor form of IL-18 (pro IL-18) is cleaved by caspase-1 resulting in a mature protein that is biologically active.

IL-12 is a heterodimer comprised of two subunits p40 and p35 which are covalently linked by a disulfide bond to form an active molecule. Simultaneous expression of the two subunits is necessary for the production of the biologically active heterodimer. Both human and murine p35 and p40 IL-12 single chain proteins (i.e., a single protein containing both p35 and p40 subunits) have been produced; see e.g., Lieschke et al., 1997, *Nature Biotechnology* 15, 35-40. Co-expression of the human and murine p35 and p40 cDNA subunits of IL-12 resulting in a biologically active IL-12 heterodimer was achieved by Gubler et al., 1991, *Proc. Natl. Acad. Sci.* U.S.A., 88, 4143-4147 and Schoenhaut et al., 1992, *J. Immunol.*, 148, 3433-3440, respectively. cDNAs encoding canine IL-12 p35 and p40 subunits were isolated and co-transfected to express canine IL-12 by Okano et al., 1997, *J. Interferon Cytokine Res.* 17, 713-718. cDNAs encoding feline p35 and p40 subunits have been isolated and expressed; see, for example, Fehr et al., 1997, *DNA Seq.* 8, 77-82; Schijns et al., 1997, *Immunogenetics* 45,

462-463; Bush et al., 1994, *Molec. Immunol.* 31, 1373-1374. At the amino acid level, canine and feline IL-12 p40 subunit share 92.7 percent identity to each other; share 84.8 and 84.2 percent identity to human IL-12 p40, respectively; and share 67.4 and 68.9 percent identity to murine IL-12 p40, respectively. IL-12 shares some biological activities with IL-18 including IFN-γ production in T cells. IL-18 and IL-12 in combination work synergistically to increase IFN-γ production in T cells; as such these cytokines when utilized alone or in combination can be very effective in mediating IgE responses.

Caspase-1 may play a key role in the processing of IL-18 precursor in cells where IL-18 is produced. It may be that coexpression of caspase-1 along with IL-18 may be necessary for the proper processing of the IL-18 precursor and enhanced secretion of the processed IL-18 mature polypeptide. A cDNA encoding equine caspase-1 was isolated by Wardlow, et al. (unpublished; direct submission to GenBank, accession number AF090119). Feline caspase-1 cDNA has a 71% homology to equine caspase-1 cDNA and feline caspase-1 protein has a 48.8% homology to equine caspase-1 protein. A cDNA encoding equine caspase-1 was isolated by Cerretti, et al. (Science 256, p 97-100 (1992); GenBank accession number M87507). Feline caspase-1 cDNA has a 60% homology to human caspase-1 cDNA and feline caspase-1 protein has a 60% homology to human caspase-1 protein. A cDNA encoding rat caspase-1 (called interleukin-1 beta converting enzyme) was isolated by Keane, et al. (Cytokine 7(2) 105-110 1995); GenBank accession number U14647). Feline caspase-1 cDNA has a 55.4% homology to rat caspase-1 cDNA and feline caspase-1 protein has a 40.2% homology to rat caspase-1 protein. A cDNA encoding murine caspase-1 was isolated by Molineaux, et al. (Proc Natl. Acad. Sci 90,

1809-1813, 1993); GenBank accession number L28095). Feline caspase-1 cDNA has a 55.7% homology to murine caspase-1 cDNA and feline caspase-1 protein has a 38.5% homology to murine caspase-1 protein. A cDNA encoding canine caspase-1 was isolated by Taylor, et al. (2000) *DNA Seq.* 10(6), pp 387-394; GenBank accession number AF135967). Feline caspase-1 cDNA has a 90% homology to canine caspase-1 cDNA.

To date, however, neither IL-18 nor caspase-1, nor the nucleic acid molecules encoding such proteins, have been isolated from cats. Neither have IL-12 single chain proteins been produced using feline or canine IL-12 subunits. As such there remains a need for compounds and methods to regulate an immune response in cats and dogs through manipulation of IL-18, caspase-1 and IL-12 single chain activities.

SUMMARY OF THE INVENTION

The present invention relates to canine and feline proteins, nucleic acid molecules encoding such proteins, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. In a preferred embodiment, the present invention relates to feline interleukin-18 (IL-18), feline caspase-1 (casp-1), feline interleukin-12 (IL-12) single chain and canine interleukin-12 single chain proteins, nucleic acid molecules, antibodies and inhibitors. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies and/or inhibitors, as well as their use to evaluate and regulate an immune response in an animal.

One embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) an isolated nucleic acid molecule selected from the group consisting of (i) a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ

ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13; and (ii) a nucleic acid molecule comprising at least 70 contiguous nucleotides identical in sequence to at least 70 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, and SEQ ID NO:41; (b) an isolated nucleic acid molecule selected from the group consisting of (i) a nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:25, and (ii) a nucleic acid molecule comprising at least 70 contiguous nucleotides identical in sequence to at least 70 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:25; (c) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising ((a)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:29, and a nucleic acid sequence comprising at least 44 contiguous nucleotides identical in sequence to at least 44 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:26 and SEQ ID NO:29; ((b)) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60; and ((c)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:35, and a nucleic acid molecule comprising at least 44 contiguous nucleotides identical in sequence to at least 44 contiguous nucleotides of a nucleic acid sequence selected from the group consisting

of SEQ ID NO:32 and SEQ ID NO:35, such that said nucleic acid molecule of (i) encodes a feline IL-12 single chain protein; and (ii) a nucleic acid molecule comprising a nucleic acid sequence fully complementary to the coding strand of any of said nucleic acid molecules as set forth in (i); (d) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising ((a)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:58, and a nucleic acid sequence comprising at least 47 contiguous nucleotides identical in sequence to at least 47 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:49; ((b)) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60; and ((c)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:46, SEQ ID NO:49, and a nucleic acid molecule comprising at least 47 contiguous nucleotides identical in sequence to at least 47 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:49, such that said nucleic acid molecule of (i) encodes a canine IL-12 single chain protein; and (ii) a nucleic acid molecule comprising a nucleic acid sequence fully complementary to the coding strand of any of said nucleic acid molecules as set forth in (i); (e) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule having a nucleic acid sequence that is at least 92 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13; and (ii) a nucleic acid molecule comprising a fragment of a nucleic acid molecule of (i) wherein said fragment is at least

80 nucleotides in length; (f) an isolated nucleic acid molecule selected from the group consisting of (i) a nucleic acid molecule having a nucleic acid sequence that is at least 85 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23 and SEQ ID NO:25, and (ii) a nucleic acid molecule comprising a fragment of a nucleic acid molecule of (i) wherein said fragment is at least 85 nucleotides in length; (g) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising ((a)) a nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:26 and SEQ ID NO:29, or a fragment thereof of at least 55 nucleotides in length; ((b)) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60; and ((c)) nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:35, or a fragment thereof of at least 55 nucleotides in length, such that said nucleic acid molecule (i) encodes a feline IL-12 single chain protein; and (ii) a nucleic acid molecule comprising a nucleic acid sequence fully complementary to the coding strand of a nucleic acid molecule as set forth in (i); and (h) an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising ((a)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:58, and a nucleic acid sequence comprising at least 55 contiguous nucleotides identical in sequence to at least 55 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of NO:52 and SEQ ID NO:58; ((b)) a nucleic acid linker of $(XXX)_n$ wherein

n=0 to 60; and ((c)) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:46, SEQ ID NO:49, and a nucleic acid molecule comprising at least 55 contiguous nucleotides identical in sequence to at least 55 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:35, such that said nucleic acid molecule of (i) encodes a canine IL-12 single chain protein; and (ii) a nucleic acid molecule comprising a nucleic acid sequence fully The present invention also includes recombinant molecules, recombinant viruses and recombinant cells comprising such IL-18, caspase-1, and IL-12 single chain nucleic acid molecules and methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (a) a nucleic acid molecule having a nucleic acid sequence encoding an IL-18 protein selected from the group consisting of: (i) a protein selected from the group consisting of ((a)) a protein having an amino acid sequence that is at least 92 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12, and ((b)) a protein comprising a fragment of a protein of ((a)), wherein said fragment is at least 30 amino acids in length; and (ii) a protein comprising at least 25 contiguous amino acids identical in sequence to at least 25 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12; (b) a nucleic acid molecule having a nucleic acid sequence encoding a caspase-1 protein selected from the group consisting of: (i) a protein selected from the group consisting of ((a)) a protein having an amino acid sequence that is at least 85 percent

identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and ((b)) a protein comprising a fragment of a protein of ((a)), wherein said fragment is at least 30 amino acids in length; and (ii) a protein comprising at least 25 contiguous amino acids identical in sequence to at least 25 contiguous amino acids selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24; (c) a nucleic acid molecule having a nucleic acid sequence encoding an IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to a IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p40 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30 and wherein said p35 subunit domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p35 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36; (d) a nucleic acid molecule having a nucleic acid sequence encoding an IL-12

single chain protein comprising an IL-12 p40 subunit domain linked to a IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p40 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, and wherein said p35 subunit domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p35 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50; (e) a nucleic acid molecule comprising a nucleic acid sequence fully complementary to the coding strand of any of said nucleic acid molecules as set forth in (a), (b), (c), or (d). The present invention also includes recombinant molecules, recombinant viruses and recombinant cells comprising such IL-18, caspase-1, and IL-12 single chain nucleic acid molecules and methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention is an isolated protein selected from the group consisting of: (a) an isolated IL-18 protein selected from the group consisting of: (i) an isolated protein of at least 25 amino acids in length, wherein said protein has an at least 25 contiguous amino acid region identical in sequence to a 25 contiguous amino acid region selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12; and (ii) an isolated protein having an amino acid sequence that is at least 92 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12 and a fragment thereof of at least 30 nucleotides; wherein said isolated protein has a function selected from the group consisting of (i) eliciting an immune response against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12, (ii) selectively binding to an antibody raised against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and (iii) exhibiting IL-18 activity; (b) an isolated caspase-1 protein selected from the group consisting of: (i) an isolated protein of at least about 25 amino acids in length, wherein said protein has an at least 25 contiguous amino acid region identical in sequence to a 25 contiguous amino acid region selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24; and (ii) an isolated protein having an amino acid sequence that is at least 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24 and has a nucleic acid fragment thereof of at least 30 nucleotides; wherein said isolated protein has a function selected from the group

consisting of (i) eliciting an immune response against a caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, (ii) ii) selectively binding to an antibody raised against caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and (iii) exhibiting caspase-1 activity; (c) an isolated IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to an IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p40 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30 and wherein said p35 subunit domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p35 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36; wherein said isolated protein has a function selected from the group consisting of (i) eliciting an immune response against an IL-12 protein having an amino

acid sequence selected from the group consisting of SEQ ID NO:39 and SEQ ID NO:44, (ii) selectively binding to an antibody raised against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67, and (iii) exhibiting IL-12 activity; and (d) an isolated IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to an IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p40 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59; wherein said p35 subunit domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p35 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50; and wherein said isolated protein has a function selected from the group

consisting of (i) eliciting an immune response against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:62, and SEQ ID NO:67, (ii) selectively binding to an antibody raised against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67, and (iii) exhibiting IL-12 activity. The present invention also includes recombinant molecules, recombinant viruses and recombinant cells comprising such IL-18, caspase-1, and IL-12 single chain nucleic acid molecules and methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

The present invention also includes an antibody that selectively binds to a protein of the present invention as well as methods to produce and use such proteins or antibodies. By selectively is meant an antibody that binds to a protein of the present invention, but does not bind a similar protein of another species.

One aspect of the present invention is a therapeutic composition that, when administered to an animal, regulates an immune response in the animal. Such a therapeutic composition includes at least one of the following protective compounds: an IL-18, caspase-1, or IL-12 single chain protein of the present invention, a mimetope of any of the proteins, a multimeric form of any of the proteins, an isolated nucleic acid molecule of the present invention, an antibody that selectively binds any of the proteins, and/or an inhibitor of a protein activity identified by its ability to inhibit the activity of

any of the proteins. Also included is a method to regulate an immune response by administering such a therapeutic composition to an animal.

The present invention also includes a method to produce a protein of the present invention; such a method includes the step of culturing a recombinant cell capable of expressing a protein being encoded by a nucleic acid molecule of the present invention.

Another embodiment of the present invention is a method to identify a compound capable of regulating an immune response in an animal, a method selected from the group consisting of : (a) contacting an isolated feline IL-18 protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has T cell stimulating activity inducing T cells to make interferon gamma, and determining if the putative inhibitory compound inhibits that activity; (b) contacting an isolated feline caspase-1 protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein cleaves precursor form of IL-18 resulting in biologically active IL-18, and determining if the putative inhibitory compound inhibits that activity; and (c) contacting an isolated IL-12 single chain protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has T cell proliferation stimulating activity, and determining if the putative inhibitory compound inhibits that activity.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated feline and canine proteins, nucleic acid molecules encoding such proteins, antibodies raised against such proteins and/or inhibitors of such proteins or nucleic acid molecules. Specifically, the present invention provides for isolated feline IL-18, feline caspase-1, and feline and canine IL-12 single

chain proteins and nucleic acid molecules as well as antibodies raised against such proteins, and/or inhibitors of such proteins or nucleic acid molecules. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies, inhibitors and/or other compounds derived therefrom as therapeutic compositions to regulate the immune response of an animal as well as in other applications, such as those disclosed below.

One embodiment of the present invention is an isolated protein that includes a feline IL-18 protein, a feline caspase-1 protein, a feline IL-12 single chain protein and/or a canine IL-12 single chain protein. As used herein, a feline and/or canine protein refers to a protein. As used herein, a protein of the present invention is a protein that is isolated from a felid or a canid or is derived therefrom and can be produced by methods known in the art, such as, for example, using recombinant DNA technology or by chemical synthesis. As such, a feline or canine protein of the present invention includes natural forms as well as any variants thereof, such as a feline or canine protein that has been altered in a manner known to those skilled in the art, such as those methods disclosed herein. As used herein, a feline or canine protein does not refer to a mouse or human protein.

Similarly, a feline or canine nucleic acid molecule of the present invention includes a feline IL-18 nucleic acid molecule, a feline caspase-1 nucleic acid molecule, a feline IL-12 single chain nucleic acid molecule and/or canine IL-12 single chain nucleic acid molecule. As used herein a feline or canine nucleic acid molecule of the present invention refers to a nucleic acid molecule that includes a nucleic acid molecule that encodes a protein of the present invention and/or a complement thereof. As such, a feline

IL-18 nucleic acid molecule, a feline caspase-1 nucleic acid molecule, a feline IL-12 single chain nucleic acid molecule or a canine IL-12 single chain nucleic acid molecule of the present invention is a nucleic acid molecule that encodes a feline IL-18 protein, a feline caspase-1 protein, a feline IL-12 single chain protein or a canine IL-12 single chain protein, respectively, and/or that is a complement thereof. As used herein, a feline or canine nucleic acid molecule of the present invention is a nucleic acid molecule that is isolated from a felid or canid or is derived therefrom and can be produced using methods known in the art, such as, for example, recombinant DNA technology, or by chemical synthesis. As such, a feline or canine nucleic acid molecule of the present invention includes natural forms as well as any variants thereof, such as a feline or canine nucleic acid molecule that has been altered in a manner known to those skilled in the art, such as those methods disclosed herein. As used herein, a feline or canine nucleic acid molecule does not refer to a mouse or human nucleic acid molecule.

According to the present invention, an isolated, or biologically pure, nucleic acid molecule or protein, is a nucleic acid molecule or protein that has been removed from its natural milieu. As such, "isolated" and/or "biologically pure" do not necessarily reflect the extent to which the nucleic acid molecule or protein has been purified. "Proteins" are defined as any compounds which comprise amino acids, including peptides, polypeptides and fusion proteins. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, an item "selected from the

group consisting of" refers to one or more of the items in that group, including combinations thereof. The term "fragment " refers to any subset of the referent nucleic acid molecule. Furthermore, the term "linked in frame" refers to nucleic acid fragment joined to another nucleic acid fragment in a manner such that the molecule is able to be expressed when transformed into a host cell.

As used herein, a felid refers to any member of the felid family (i.e. the family Felidae), including, but not limited to, domestic cats, and wild cats such as tigers, lions, and lynx. Similarly, the term feline refers to "of the family Felidae".

As used herein, a canid refers to any member of the canid family (i.e. the family Canidae), including, but not limited to, domestic dogs, and wild canids such as wolves, foxes, and coyotes. Similarly, the term canine refers to "of the family Canidae".

Nucleic acid molecules of the present invention of known length isolated from *Felis catus* are denoted as follows: a feline IL-18 nucleic acid molecule is denoted as nFeIL-18_x, wherein "x" refers to the number of nucleotides in that molecule; for example, nFeIL-18_{607} refers to a feline IL-18 nucleic acid molecule of 607 nucleotides in length; and in a similar fashion, a feline Casp-1 nucleic acid molecule of length "x" is referred to as nFeCasp-1_x, a feline IL-12 single chain nucleic acid molecule of length "x" is referred to as nFeIL-12_x, a feline IL-12p35 subunit nucleic acid molecule of length "x" is referred to as nFeIL-$12p35_x$ and a feline IL-12p40 subunit nucleic acid molecule of length "x" is referred to as nFeIL-$12p40_x$. Similarly, *Felis catus* IL-18, Casp-1, IL-12 single chain, IL-12p35 subunit, and IL-12p40 subunit proteins of the present invention of known length are denoted PFeIL-18_x, PFeCasp-1_x, PFeIL-12_x, PFeIL-$12p35_x$, and PFeIL-$12p40_x$ respectively.

Nucleic acid molecules of the present invention of known length isolated from *Canis familiaris* are denoted as follows: a canine IL-12 single chain is denoted as nCaIL-12_x, wherein "x" refers to the number of nucleotides in that molecule; for example, nCaIL-12_{1602} refers to a canine IL-12 single chain nucleic acid molecule of 1602 nucleotides in length and in a similar fashion, a canine IL-12 single chain nucleic acid molecule of length "x" is referred to as nCaIL-$12p35_x$ and a canine IL-12p40 subunit nucleic acid molecule of length "x" is referred to as nCaIL-$12p40_x$. Similarly, *Canis familiaris* IL-12 single chain proteins of the present invention of known length isolated from are denoted PCaIL-12_x, PCaIL-$12p35_x$, or PCaIL-$12p40_x$ respectively. The present invention includes nucleic acid molecules selected from the group consisting of nCaIL-$12p35_{591}$, nCaIL-$12p40_{2267}$, nCaIL-$12p40_{1002}$, nCaIL-$12p40_{987}$, nCaIL-12_{1599}, and nCaIL-12_{1533}.

The present invention includes nucleic acid molecules that include one or more of the following nucleic acid sequences: SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, SEQ ID NO:23, SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:38, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:46, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:58, SEQ ID NO:61, SEQ ID NO:64 and/or SEQ ID NO:66, and/or a complements of these nucleic acid sequences, i.e. SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:13, SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:22, SEQ ID NO:25, SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:34, SEQ ID NO:37, SEQ ID NO:40, SEQ ID NO:45, SEQ ID NO:48, SEQ ID NO:51, SEQ ID NO:54, SEQ ID NO:60, SEQ ID NO:63, and/or SEQ ID NO:68, respectively. Complements are defined

as two single strands of nucleic acid in which the nucleotide sequences are such that the strands will hybridize as a result of base pairing throughout their full length; i.e., these sequences are fully complementary. Such nucleic acid sequences are further described herein and can be easily be determined by those skilled in the art. It should be noted that since nucleic acid sequencing technology is not entirely error-free, nucleic acid and protein sequences presented herein represent apparent nucleic acid and amino acid sequences of the isolated nucleic acid molecules and proteins, respectively, of the present invention.

As used herein, an isolated feline IL-18, feline caspase-1, feline IL-12p40 subunit, feline IL-12p35 subunit, feline IL-12 single chain, canine IL-12p40 subunit, canine IL-12p35, and/or canine IL-12 single chain protein of the present invention can be a full-length protein or any homolog of such a protein, including truncated forms of the protein. An isolated IL-18 protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against, (or to) an IL-18 protein, whether the protein has IL-18 activity, such as T cell stimulating activity, or selectively binding to an antibody raised against an IL-18 protein. An isolated caspase-1 protein of the present invention may be identified in a straight-forward manner by the protein's ability to elicit an immune response against, (or to) a caspase-1 protein, whether the protein has caspase-1 activity, such as cleaving the precursor form of IL-18 resulting in a biologically active IL-18, or selectively binding to an antibody raised against a caspase-1 protein. A IL-12 single chain protein of the present invention may be identified in a straight-forward manner by the protein's ability to elicit an immune response against, (or to) an IL-12 protein, including the p35 or p40 subunits, whether the

protein has IL-12 activity, such as T cell stimulating activity, or selectively binding to an antibody raised against an IL-12 protein, including the p35 or p40 subunits. Examples of protein homologs of the present invention includes proteins of the present invention in which amino acids have been deleted (e.g. a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g. by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the protein homolog include at least one epitope capable of eliciting an immune response against the parent protein, where the term parent refers to the longer and/or full-length protein that the homolog is derived from. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art. As used herein, the term "epitope" refers to the smallest portion of a protein capable of selectively binding to the antigen binding site of an antibody. It is well accepted by those skilled in the art that the minimal size of a protein epitope capable of selectively binding to the antigen binding site of an antibody is about five or six to seven amino acids.

Proteins of the present invention include variants of a full-length protein of the present invention. Such variants include proteins that are less than full-length. As used herein, variants of the present invention refer to nucleic acid molecules that are naturally-occurring a defined below, and may result from alternative RNA splicing, alternative termination of an amino acid sequence or DNA recombination. Examples of variants include allelic variants as defined below. It is to be noted that a variant is an example of a homolog of the present invention.

Proteins of the present invention are encoded by nucleic acid molecules of the present invention. As used herein, an IL-18 nucleic acid molecule includes sequences related to a natural feline IL-18 gene. As used herein, a caspase-1 protein includes nucleic acid sequences related to a natural feline caspase-1 gene. As used herein, an IL-12 single chain nucleic acid molecule includes sequences related to a natural canine or feline IL-12 gene, IL-12p35 gene, and/or IL-12p40 gene. As used herein, a feline IL-18, a feline caspase-1, and or a feline or canine IL-12 single chain refers to the natural genomic elements that encode a feline IL-18, a feline caspase-1, and or a feline or canine IL-12 single chain, and includes all regions such as regulatory regions that control production of the protein encoded by the gene) such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a sequence may include that sequence in one continuous array, or may include the sequence of fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that region that is translated into a full-length, i.e., a complete protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment of the present invention, isolated proteins are encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to the non-coding strand of nucleic acid molecules encoding proteins. The minimal size of a protein of the present invention (4-6 amino acids) is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid, i.e., hybridizing under stringent hybridization conditions, with the complementary sequence of a nucleic acid molecule

encoding the corresponding natural protein. The size of a nucleic acid molecule encoding such a protein is dependent on the nucleic acid composition and the percent homology between the nucleic acid molecule and the complementary nucleic acid sequence. It can easily be understood that the extent of homology required to form a stable hybrid under stringent conditions can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered, i.e. localized, in distinct regions on a given nucleic acid molecule.

The minimal size of a feline IL-18, feline caspase-1, and/or canine or feline IL-12 single chain protein homolog/portion/fragment of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e. hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. Stringent hybridization conditions are determined based on defined physical properties of the gene to which the nucleic acid molecule is being hybridized, and can be defined mathematically. Stringent hybridization conditions are those experimental parameters that allow an individual skilled in the art to identify significant similarities between heterologous nucleic acid molecules. These conditions are well known to those skilled in the art. See, for example, Sambrook, *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press, and Meinkoth, *et al.*, 1984, *Anal. Biochem. 138*, 267-284, each of which is incorporated herein by this reference. As explained in detail in the cited references, the determination of hybridization conditions involves the manipulation of a set of variables including the ionic strength (M, in moles/liter), the hybridization temperature (°C), the concentration of nucleic acid helix destabilizing

agents, such as formamide, the average length of the shortest hybrid duplex (n), and the percent G + C composition of the fragment to which an unknown nucleic acid molecule is being hybridized. For nucleic acid molecules of at least about 150 nucleotides, these variables are inserted into a standard mathematical formula to calculate the melting temperature, or T_m, of a given nucleic acid molecule. As defined in the formula below, T_m is the temperature at which two complementary nucleic acid molecule strands will disassociate, assuming 100% complementarity between the two strands:

$$T_m = 81.5^{\circ}C + 16.6 \log M + 0.41(\%G + C) - 500/n - 0.61(\%\text{formamide}).$$

For nucleic acid molecules smaller than about 50 nucleotides, hybrid stability is defined by the dissociation temperature (T_d), which is defined as the temperature at which 50% of the duplexes dissociate. For these smaller molecules, the stability at a standard ionic strength is defined by the following equation:

$$T_d = 4(G + C) + 2(A + T).$$

A temperature of 5°C below T_d is used to detect hybridization between perfectly matched molecules.

Also well known to those skilled in the art is how base pair mismatch, i.e. differences between two nucleic acid molecules being compared, including non-complementarity of bases at a given location, and gaps due to insertion or deletion of one or more bases at a given location on either of the nucleic acid molecules being compared, will affect T_m or T_d for nucleic acid molecules of different sizes. For example, T_m decreases about 1°C for each 1% of mismatched base pairs for hybrids greater than about 150 bp, and T_d decreases about 5°C for each mismatched base pair for hybrids below about 50 bp. Conditions for hybrids between about 50 and about 150 base pairs can be

determined empirically and without undue experimentation using standard laboratory procedures well known to those skilled in the art. These simple procedures allow one skilled in the art to set the hybridization conditions, by altering, for example, the salt concentration, the formamide concentration or the temperature, so that only nucleic acid hybrids with greater than a specified % base pair mismatch will hybridize. Stringent hybridization conditions are commonly understood by those skilled in the art to be those experimental conditions that will allow about 30% or less base pair mismatch, i.e., at least about 85% identity. Because one skilled in the art can easily determine whether a given nucleic acid molecule to be tested is less than or greater than about 50 nucleotides, and can therefore choose the appropriate formula for determining hybridization conditions, he or she can determine whether the nucleic acid molecule will hybridize with a given gene or specified nucleic acid molecule under stringent hybridization conditions and similarly whether the nucleic acid molecule will hybridize under conditions designed to allow a desired amount of base pair mismatch.

Hybridization reactions are often carried out by attaching the nucleic acid molecule to be hybridized to a solid support such as a membrane, and then hybridizing with a labeled nucleic acid molecule, typically referred to as a probe, suspended in a hybridization solution. Examples of common hybridization reaction techniques include, but are not limited to, the well-known Southern and northern blotting procedures. Typically, the actual hybridization reaction is done under non-stringent conditions, i.e., at a lower temperature and/or a higher salt concentration, and then high stringency is achieved by washing the membrane in a solution with a higher temperature and/or lower salt concentration in order to achieve the desired stringency.

In one embodiment, an IL-18 gene of the present invention includes the nucleic acid molecule SEQ ID NO:1, as well as the complement of SEQ ID NO:1. Nucleic acid sequence SEQ ID NO:1 represents the deduced sequence of the coding strand of a cDNA (complementary DNA) denoted herein as nucleic acid molecule nFeIL-18-N_{514}, the production of which is disclosed in the Examples. SEQ ID NO:1 comprises an apparent partial coding region of nFeIL-18, coding for the N-terminal portion of feline IL-18 protein. The complement of SEQ ID NO:1 (represented herein by SEQ ID NO:3) refers to the nucleic acid sequence of the strand fully complementary to the strand having SEQ ID NO:1, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence complement of any nucleic acid sequence of the present invention tat is fully complementary (i.e. can form a double helix with) the strand for which the sequence is cited. It should be noted that since nucleic acid sequencing technology is not entirely error-free, SEQ ID NO:1 (as well as other nucleic acid and protein sequences presented herein) represents an apparent nucleic acid sequence of the nucleic acid molecule encoding an immunoregulatory protein of the present invention.

Another IL-18 gene of the present invention includes the nucleic acid molecule SEQ ID NO:4, as well as the complement represented by SEQ ID NO:6. Nucleic acid sequence SEQ ID NO:4 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-18-C_{502}, the production of which is disclosed in the examples. Nucleic acid nFeIL-18-C_{502} represents an apparent partial coding region of FeIL-18, encoding a partial C-terminal region of the feline IL-18 protein. Another IL-18 gene of the present invention includes the nucleic acid molecule SEQ ID

NO:7, as well as the complement represented by SEQ ID NO:10. Nucleic acid sequence SEQ ID NO:7 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-18_{607}, the production of which is disclosed in the examples. Nucleic acid nFeIL-18_{607} represents an apparent full-length coding region of the feline IL-18 protein. Another IL-18 gene of the present invention includes the nucleic acid molecule SEQ ID NO:9, as well as the complement represented by SEQ ID NO:41. Nucleic acid sequence SEQ ID NO:9 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFe IL-18_{576}, the production of which is disclosed in the examples. Nucleic acid molecule nFe IL-18_{576} represents the coding region for an apparent precursor protein to a mature feline IL-18 protein. Another IL-18 gene of the present invention includes the nucleic acid molecule SEQ ID NO:11, as well as the complement represented by SEQ ID NO:13. Nucleic acid sequence SEQ ID NO:11 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFe IL-18_{471}, the production of which is disclosed in the Examples. Nucleic acid molecule nFe IL-18_{471} represents the coding region for an apparent mature IL-18 protein. The putative cleavage site for the mature IL-18 protein is between amino acid positions 35 and 36 of SEQ ID NO:8, representing PFeIL-18_{192}, which is the predicted amino acid sequence of the full-length IL-18 protein (i.e., containing signal, or leader, peptide). SEQ ID NO:12 represents the predicted amino acid sequence of the mature IL-18 protein (i.e., without the signal, or leader, sequence), also denoted as PFeIL-18_{157}.

In another embodiment, a caspase-1 gene of the present invention includes the nucleic acid sequence SEQ ID NO:14, as well as the complement represented by SEQ ID

NO:16. Nucleic acid sequence SEQ ID NO:14 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeCasp-1_{1233}, the production of which is disclosed in the Examples. Nucleic acid molecule nFeCasp-1_{1233} represents the coding region for an apparent full-length feline caspase-1 protein and includes a human primer sequence. Another caspase-1 protein of the present invention includes the nucleic acid sequence SEQ ID NO:17, as well as the complement represented by SEQ ID NO:19. Nucleic acid sequence SEQ ID NO:17 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeCasp-1-N_{526}, the production of which is disclosed in the Examples. Nucleic acid molecule nFeCasp-1-N_{526} represents the coding region for the apparent N-terminal region of the feline caspase-1 protein. Another caspase-1 protein of the present invention includes the nucleic acid molecule SEQ ID NO:20, as well as the complement represented by SEQ ID NO:22. Nucleic acid sequence SEQ ID NO:20 represents the deduced sequence of a coding strand of a cDNA denoted herein as nucleic acid molecule nFeCasp-1-C_{500}, the production of which is disclosed in the Examples. Nucleic acid molecule nFeCasp-1-C_{500} represents the coding region for the apparent C-terminal region of the feline caspase-1 protein. Another caspase-1 protein of the present invention includes the nucleic acid molecule SEQ ID NO:23, as well as the complement represented by SEQ ID NO:25. Nucleic acid sequence SEQ ID NO:23 represents the deduced sequence of a coding strand of a cDNA denoted herein as nucleic acid molecule nFeCasp-1_{1230}, the production of which is disclosed in the examples. Nucleic acid molecule nFeCasp-1_{1230} represents the coding region for the apparent full-length feline caspase-1 protein, denoted herein as PFeCasp-1_{410}, represented herein as SEQ ID NO:24.

In another embodiment, feline IL-12 single chain proteins of the present invention contain both a mature IL-12 p35 subunit and a full-length IL-12 p40 subunit, joined by a linker. An IL-12 single chain gene of the present invention includes the nucleic acid sequence SEQ ID NO:38, as well as the complement represented by SEQ ID NO:40. Nucleic acid sequence SEQ ID NO:38 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12_{1599}, the production of which is disclosed in the Examples. Nucleic acid molecule nFeIL-12_{1599} represents the coding region encoding a single chain full-length feline IL-12 protein, which includes the coding region for a full-length (i.e. containing signal, or leader, sequence) IL-12 p40 subunit, a linker of the present invention, and the coding region for a mature (i.e. not containing signal, or leader, sequence) IL-12 p35 subunit. SEQ ID NO:38 comprises a sequence that includes both the nucleic acid sequence SEQ ID NO:29 (nucleic acid sequence SEQ ID NO:29 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p40_{987}, which represents the coding region encoding the full-length feline IL-12 p40 subunit, whereas SEQ ID NO:31 represents the complement of SEQ ID NO:29) and SEQ ID NO:35 (nucleic acid sequence SEQ ID NO:35 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p35_{591}, which represents the coding region encoding the mature feline IL-12 p35 subunit, whereas SEQ ID NO:37 represents the complement of SEQ ID NO:35). Translation of SEQ ID NO:38 yields a predicted protein denoted herein as PFeIL-12_{533}, also denoted as SEQ ID NO:39.

In another embodiment, feline IL-12 single chain proteins of the present invention contain both a mature IL-12 p35 subunit and an mature IL-12 p40 subunit, joined by a

linker. An IL-12 single chain gene of the present invention includes the nucleic acid sequence SEQ ID NO:43, as well as the complement represented by SEQ ID NO:45. Nucleic acid sequence SEQ ID NO:43 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12_{1533}, the production of which is disclosed in the Examples. Nucleic acid molecule nFeIL-12_{1533} represents the coding region encoding a single chain mature feline IL-12 protein. SEQ ID NO:33 comprises a sequence that includes both the nucleic acid sequence SEQ ID NO:26 (nucleic acid sequence SEQ ID NO:26 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p40_{985}, which represents the coding region encoding the mature feline IL-12 p40 subunit, whereas SEQ ID NO:28 represents the complement of SEQ ID NO:26) and SEQ ID NO:35 (nucleic acid sequence SEQ ID NO:35 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p35_{591}, which represents the coding region encoding the mature feline IL-12 p35 subunit, whereas SEQ ID NO:37 represents the complement of SEQ ID NO:35). Translation of SEQ ID NO:43 yields a predicted protein denoted herein as PFeIL-12_{511}, also denoted as SEQ ID NO:44.

In another embodiment, canine IL-12 single chain proteins and nucleic acid molecules of the present invention contain both a mature IL-12 p35 subunit and a full-length IL-12 p40 subunit, joined by a linker. An IL-12 single chain gene of the present invention includes the nucleic acid sequence SEQ ID NO:61, as well as the complement represented by SEQ ID NO:63. Nucleic acid sequence SEQ ID NO:61 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaIL-12_{1599}, the production of which is disclosed in the Examples. Nucleic

acid molecule nCaIL-12_{1599} represents the coding region encoding a single chain full-length canine IL-12 protein, which includes the coding region for a full-length (i.e. containing signal, or leader, sequence), IL-14 p40 subunit, a linker of the present invention, and the coding region for a mature, (i.e., not containing signal, or leader, sequence) IL-12 p35 subunit. SEQ ID NO:61 comprises a nucleic acid sequence that includes both the nucleic acid sequence SEQ ID NO:58 (nucleic acid SEQ ID NO:58 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaIL-12_{987}, which represents the coding region encoding the full-length canine IL-12 p40 subunit, whereas SEQ ID NO:60 represents the complement of SEQ ID NO:58) and SEQ ID NO:49 (nucleic acid sequence SEQ ID NO:49 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p35_{591}, which represents the coding region encoding the mature canine IL-12 subunit, whereas SEQ ID NO:51 represents the complement of SEQ ID NO:49. Translation of SEQ ID NO:61 yields a predicted protein denoted herein as PCaIL-12_{533}, also denoted as SEQ ID NO:62.

In another embodiment, canine IL-12 single chain proteins and nucleic acid molecules of the present invention contain both a mature IL-12 p35 subunit and a mature IL-12 p40 subunit, joined by a linker. An IL-12 single chain gene of the present invention includes the nucleic acid sequence SEQ ID NO:66, as well as the complement represented by SEQ ID NO:68. Nucleic acid sequence SEQ ID NO:66 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaIL-12_{1533}, the production of which is disclosed in the Examples. Nucleic acid molecule nCaIL-12_{1533} represents the coding region encoding a single chain full-

length canine IL-12 protein, which includes the coding region for a full-length (i.e. containing signal, or leader, sequence), IL-14 p40 subunit, a linker of the present invention, and the coding region for a mature, (i.e., not containing signal, or leader, sequence) IL-12 p35 subunit. SEQ ID NO:66 comprises a nucleic acid sequence that includes both the nucleic acid sequence SEQ ID NO:52 (nucleic acid SEQ ID NO:52 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nCaIL-12_{921}, which represents the coding region encoding the mature canine IL-12 p40 subunit, whereas SEQ ID NO:54 represents the complement of SEQ ID NO:52) and SEQ ID NO:49 (nucleic acid sequence SEQ ID NO:49 represents the deduced sequence of the coding strand of a cDNA denoted herein as nucleic acid molecule nFeIL-12p35_{591}, which represents the coding region encoding the mature canine IL-12 subunit, whereas SEQ ID NO:51 represents the complement of SEQ ID NO:49. Translation of SEQ ID NO:66 yields a predicted protein denoted herein as PCaIL-12_{511}, also denoted as SEQ ID NO:67.

Nucleic acid molecules and proteins of the present invention having specific sequence identifiers are described in Table 1.

Table 1. Sequence identification numbers (SEQ ID NOs) and their corresponding nucleic acid molecule or proteins.

SEQ ID NO:	description
1	nFeIL-18-N_{514} coding strand
2	PFeIL-18-N_{133}
3	nFeIL-18-N_{514} complementary strand
4	nFeIL-18-C_{502} coding strand
5	PFeIL-18-C_{154}

SEQ ID NO:	description
6	nFeIL-18-C_{502} complementary strand
7	nFeIL-18_{607} coding strand
8	PFeIL-18_{192}
9	nFeIL-18_{576} coding strand: *coding sequence for full-length feline IL-18 protein*
10	nFeIL-18_{607} complementary strand to SEQ ID NO:7
11	nFeIL-18_{471} coding strand: *coding sequence for mature feline IL-18 protein*
12	PFeIL-18_{157}
13	nFeIL-18_{471} complementary strand
14	nFeCasp-1_{1233} coding strand
15	PFeCasp-1_{410}
16	nFeCasp-1_{1233} complementary strand
17	nFeCasp-1-N_{526} coding strand
18	PFeCasp-1-N_{169}
19	nFeCasp-1-N_{526} complementary strand
20	nFeCasp-1-C_{500} coding strand
21	PFeCasp-1-C_{120}
22	nFeCasp-1-C_{500} complementary strand
23	nFeCasp-1_{1230} coding strand: *coding sequence for feline caspase-1 protein*
24	PFeCasp-1_{410}
25	nFeCasp-1_{1230} complementary strand
26	nFeIL-12p40_{921} coding strand: *coding sequence for feline mature IL-12p40 subunit*
27	PFeIL-12p40_{307}
28	nFeIL-12p40_{921} complementary strand

SEQ ID NO:	description
29	nFeIL-12p40$_{987}$ coding strand: *coding sequence for feline full-length IL-12p40 subunit*
30	PFeIL-12p40$_{329}$
31	nFeIL-12p40$_{987}$ complementary strand
32	nFeIL-12p35$_{666}$ coding strand: *coding sequence for feline full-length IL-12p35 subunit*
33	PFeIL-12p35$_{222}$
34	nFeIL-12p35$_{666}$ complementary strand
35	nFeIL-12p35$_{591}$ coding strand: *coding sequence for feline mature IL-12p35 subunit*
36	PFeIL-12p35-N$_{187}$
37	nFeIL-12p35$_{591}$ complementary strand
38	nFeIL-12$_{1599}$ coding strand
39	PFeIL-12$_{533}$
40	nFeIL-12$_{1599}$ complementary strand
41	nFeIL-18$_{576}$ complementary strand to SEQ ID NO:9
42	not used--inactive
43	nFeIL-12$_{1533}$ coding strand
44	PFeIL-12$_{511}$
45	nFeIL-12$_{1533}$ complementary strand
46	nCaIL-12p35$_{666}$ coding strand: *coding strand for canine full-length IL-12p35 subunit*
47	PCaIL-12p35$_{222}$
48	nCaIL-12p35$_{666}$ complementary strand
49	nCaIL-12p35$_{591}$ coding strand
50	PCaIL-12p35$_{197}$
51	nCaIL-12p35$_{591}$ complementary strand

SEQ ID NO:	description
52	nCaIL-12p40$_{921}$ coding strand: *coding sequence for mature form canine IL-12 p40 subunit*
53	PCaIL-12p40$_{307}$
54	nCaIL-12p40$_{921}$ reverse complement
55	nFeIL-12p40-N$_{985}$ coding sequence
56	PCaIL-12p40-N$_{328}$
57	nFeIL-12p40-N$_{985}$ complementary strand
58	nCaIL-12p40$_{987}$ coding strand: *coding sequence for full-length canine IL-12 p40 subunit*
59	PCaIL-12p40$_{329}$
60	nCaIL-12p40$_{987}$ complementary strand
61	nCaIL-12$_{1599}$ coding strand
62	PCaIL-12$_{533}$
63	nCaIL-12$_{1599}$ complementary strand
64	not used–inactive
65	not used–inactive
66	nCaIL-12$_{1533}$ coding strand
67	PCaIL-12$_{511}$
68	nCaIL-12$_{1533}$ complementary strand
101	nFeIL-12p35-N$_{561}$ coding strand
102	PFeIL-12p35-N$_{187}$
103	nFeIL-12p35-N$_{561}$ complementary strand
104	nCaIL-12p35$_{1455}$ coding strand
105	PCaIL-12p35$_{222}$
106	nCaIL-12p35$_{1455}$ complementary strand
107	nCaIL-12p40$_{2267}$ coding strand

SEQ ID NO:	description
108	PcaIL-12p40$_{329}$
109	nCaIL-12p40$_{2267}$ complementary strand

Particularly preferred nucleic acid molecules encoding feline IL-18 proteins are nFeIL-18-N$_{514}$, nFeIL-18-C$_{502}$, nFeIL-18$_{607}$, nFeIL-18$_{576}$, and nFeIL-18$_{471}$, the coding strands of which are represented by SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:11, respectively.

Particularly preferred nucleic acid molecules encoding feline caspase-1 proteins are nFeCasp-1$_{1233}$, nFeCasp-1-N$_{526}$, nFeCasp-1-C$_{500}$ and nFeCasp-1$_{1230}$, the coding strands of which are represented by SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, and SEQ ID NO:23 respectively.

Particularly preferred nucleic acid molecules encoding canine and feline IL-12 p35 and p40 subunit proteins are nFeIL-12p40-N$_{985}$, nFeIL-12p40$_{987}$, nFeIL-12p40$_{921}$, nFeIL-12p35$_{666}$, nFeIL-12p35-N$_{561}$, nFeIL-12p35$_{591}$, nCaIL-12p35$_{666}$, nCaIL-12p35$_{1455}$, nCaIL-12p35$_{591}$, nCaIL-12p40$_{2267}$, nCaIL-12p40$_{921}$, and nCaIL-12p40$_{987}$. Coding strands of which are represented by SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:101, SEQ ID NO:104, and SEQ ID NO:107.

Additional preferred nucleic acid molecules encoding canine and feline IL-12 single chain proteins are nFeIL-12$_{1533}$, nFeIL-12$_{1599}$, nCaIL-12$_{1533}$, nCaIL-12$_{1599}$, the coding strands of which are represented by SEQ ID NO:43, SEQ ID NO:38, SEQ ID NO:61, and SEQ ID NO:66.

One embodiment of the present invention includes an isolated nucleic acid molecule that is selected from a group of nucleic acid molecules. One member of this group includes an isolated nucleic acid molecule that is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13; and a nucleic acid molecule comprising at least 70 contiguous nucleotides identical in sequence to at least 70 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13. Another member of this group of nucleic acid molecules includes an isolated nucleic acid molecule that is selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:25; and a nucleic acid molecule comprising at least 70 contiguous nucleotides identical in sequence to at least 70 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:25. Another member of this group of nucleic acid molecules includes an isolated nucleic acid molecule that is selected from the group consisting of a nucleic acid molecule comprising (a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:26, SEQ ID NO:29, and a nucleic acid sequence comprising at least 44 contiguous nucleotides identical in sequence to at least 44 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:26 and SEQ ID NO:9; (b) a nucleic

acid linker of $(XXX)_n$ wherein n=0 to 60; and (c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:32, SEQ ID NO:35, and a nucleic acid molecule comprising at least 44 contiguous nucleotides identical in sequence to at least 44 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:35, such that a nucleic acid molecule of this particular group encodes a feline IL-12 protein. Another member of this group of nucleic acid molecules includes an isolate nucleic acid molecule selected from the group consisting of a nucleic acid molecule comprising (a) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:58, and a nucleic acid sequence comprising at least 47 contiguous nucleotides identical in sequence to at least 47 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:49; (b) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60; and (c) an isolated nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO:46, SEQ ID NO:49, and a nucleic acid molecule comprising at least 47 contiguous nucleotides identical in sequence to at least 47 contiguous nucleotides of a nucleic acid sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:49, such that a nucleic acid molecule of this particular group encodes a canine IL-12 single chain protein.

The phrase, a nucleic acid molecule comprising at least "x" contiguous nucleotides identical in sequence to at least "x" contiguous nucleotides of a nucleic acid molecule selected from the group consisting of SEQ ID NO:"y", refers to an "x"-nucleotide in length nucleic acid molecule that is identical in sequence to an "x"-

nucleotide portion of SEQ ID NO:"y", as well as to nucleic acid molecules that are longer in length than "x". The additional length may be in the form of nucleotides that extend from either the 5' or the 3' end(s) of the contiguous identical "x"-nucleotide portion. The 5' and/or 3' extensions can include one or more extensions that have no identity to an immunoregulatory molecule of the present invention, as well as extensions that show similarity or identity to cited nucleic acids sequences or portions thereof.

Preferred portions, or lengths, for feline IL-18, feline caspase-1, feline IL-12 single chain, and canine IL-12 single chain nucleic acid molecules of the present invention include nucleic acid molecules of at least 40 nucleotides in length, at least 43 nucleotides in length, at least 44 nucleotides in length, at least 47 nucleotides in length, at least 50 nucleotides in length, at least 55 nucleotides in length, at least 60 nucleotides in length, at least 65 nucleotides in length, at least 70 nucleotides in length, at least 75 nucleotides in length, at least 80 nucleotides in length, at least 85 nucleotides in length, at least 90 nucleotides in length, at least 95 nucleotides in length, at least 100 nucleotides in length, at least 120 nucleotides in length, at least 140 nucleotides in length, at least 160 nucleotides in length, at least 180 nucleotides in length, at least 200 nucleotides in length, at least 250 nucleotides in length, at least 300 nucleotides in length, at least 350 nucleotides in length, at least 400 nucleotides in length, at least 450 nucleotides in length, at least 500 nucleotides in length, at least 600 nucleotides in length, at least 700 nucleotides in length, at least 800 nucleotides in length, at least 900 nucleotides in length, and a full-length molecule. Particularly preferred portions, or lengths, of the nucleic acid molecules of the present invention include nucleic acids of at least 43 nucleotides, 44 nucleotides, 47 nucleotides, 70 nucleotides, and a full length molecule.

One embodiment of a protein and/or nucleic acid molecule of the present invention is a fusion nucleic acid and/or protein that includes either a feline IL-18, caspase-1, feline IL-12 single chain, and canine IL-12 single chain nucleic acid molecule and/or protein of the present invention domain, each attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: link two or more nucleic acids and/or proteins of the present invention, to form multimeric forms of a nucleic acids and/or protein of the present invention; enhance a nucleic acid molecules or protein's stability; enhance the biological activity of a nucleic acid molecule and/or protein of the present invention; and/or assist in purification a molecule of the present invention (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, enhanced activity, and/or simplifies purification of a protein). Fusion segments can be joined to amino and/or carboxyl termini of the IL-18-containing domain, or the caspase-1 ligand-containing domain, or the IL-12 p40-containing domain, or the IL-12 p35-containing domain, or the IL-12 single chain-containing domain, of a protein and/or nucleic acid and can be susceptible to cleavage in order to enable straight-forward recovery of the protein and/or nucleic acid molecule. Fusion proteins are preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including the fusion segment attached to either the carboxyl and/or amino terminal end of a feline IL-18, feline caspase-1, feline IL-12 p35 subunit, feline IL-12 p40 subunit, feline IL-12 single chain, canine IL-12 p35 subunit, canine IL-12 p40 subunit, and/or canine IL-12 single chain-containing domain. Preferred fusion segments include a metal binding

domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of β-galactosidase, a strep tag peptide, a T7 tag peptide, a Flag™ peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra in Tampa, FL; and an S10 peptide.

The phrase, a nucleic acid linker, is a term known to those skilled in the art, and refers to a nucleic acid linker that can link, or attach, nucleic acid molecules, in such a manner that expression of the nucleic acid molecules produces one fusion protein as expression product. A linker can be any nucleotide sequence that directs expression of a single fusion polypeptide from a nucleotide molecule which includes two or more nucleic acid molecules of the present invention, wherein the fusion polypeptide has appropriate biological activity. Preferably, a nucleic acid linker of the present invention comprises nucleotides arranged in codons, (i.e., 3 nucleotides that, when transcribed, code for an amino acid residue), and the linker does not contain any stop codons in frame. A linker is represented herein as $(XXX)_n$, where X is the designation of a variable nucleotide and n refers to the number of codons. The length of the nucleic acid linker may be of any length that permits expression of the fusion protein. More preferably, the length of the nucleic acid linker is from about 0 codons to about 60 codons, or from about 0 nucleotides to about 180 nucleotides. A particularly preferred linker includes SEQ ID NO:83. Appropriate biological activity includes the ability of such a fusion protein to

elicit an immune response against a protein of the present invention, selectively binding an antibody raised against a protein of the present invention, and exhibiting the immunoregulatory activity of a protein of the present invention.

A single chain IL-12 protein of the present invention includes single chain IL-12 proteins comprising an IL-12 p35 subunit of the present invention at the N-terminus of the single chain protein and an IL-12 p40 subunit of the present invention at the C-terminus of the single chain protein, with the linker between the p35 subunit and the p40 subunit. Preferred single chain IL-12 proteins comprise an IL-12 p40 of the present invention at the N-terminus of the single chain protein and an IL-12 p40 subunit of the present invention at the C-terminus of the single chain protein, with the linker in between the subunits.

Another embodiment of the present invention includes an isolated nucleic acid molecule that is selected from the group consisting of: (i) a nucleic acid molecule having a nucleic acid sequence that is at least 92 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13; and (ii) a nucleic acid molecule comprising a fragment of a nucleic acid molecule of (i) wherein said fragment is at least 80 nucleotides in length. Preferred nucleic acid molecules include nucleic acid sequences that are at least 92 %, at least 93%, at least 94%, more preferably at least 95% identical, and even more preferably at least about 98% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13. Preferred fragment lengths include fragments of SEQ ID NO:1, SEQ ID NO:3,

SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, and SEQ ID NO:13 which are at least 75 nucleotides in length, which are at least 80 nucleotides in length, which are at least 85 nucleotides in length, which are at least 90 nucleotides in length, which are at least 100 nucleotides in length, which are at least 120 nucleotides in length, which are at least 150 nucleotides in length, which are at least 200 nucleotides in length, which are at least 300 nucleotides in length, which are at least 400 nucleotides in length, which are at least 500 nucleotides in length, which are at least 600 nucleotides in length, and which preferably are full-length.

Another embodiment of the present invention includes an isolated nucleic acid molecule that is selected from the group consisting of: (i) a nucleic acid molecule having a nucleic acid sequence that is at least 85 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:25; and (ii) a nucleic acid molecule comprising a fragment of a nucleic acid molecule of (i) wherein said fragment is at least 85 nucleotides in length. Preferred nucleic acid molecules include nucleic acid sequences that are at least 85%, preferably at least 87 %, more preferably at least 90%, even more preferably at least 95% identical to SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:25. Preferred fragment lengths include fragments of SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, and SEQ ID NO:25 which are at least 70 nucleotides in length, which are at least 80 nucleotides in length, which are at least 85 nucleotides in length, which are at least 90 nucleotides in length, which are at least 100

nucleotides in length, which are at least 200 nucleotides in length, which are at least 300 nucleotides in length, which are at least 400 nucleotides in length, which are at least 500 nucleotides in length, which are at least 600 nucleotides in length, or which preferably are full-length.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising (a) a nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:26 and SEQ ID NO:29, or a fragment thereof of at least 55 nucleotides in length; (b) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60, and (c) a nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:32 and SEQ ID NO:35, or a fragment thereof of at least 55 nucleotides in length, such that said nucleic acid molecule of (i) encodes a feline IL-12 single chain protein; and a nucleic acid molecule fully complementary to the coding strand of a nucleic acid molecule as set forth in (i). Preferred nucleic acid molecules include nucleic acid sequences that are at least 87%, at least 88%, at least 89%, more preferably at least 90%, even more preferably at least 95% identical to SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, and SEQ ID NO:35. Preferred fragment lengths include fragments of SEQ ID NO:26, SEQ ID NO:29, SEQ ID NO:32, and SEQ ID NO:35, which are at least 55 nucleotides in length, which are at least 60 nucleotides in length, which are at least about 65 nucleotides in length, which are at least 70 nucleotides in length, which are at least 80 nucleotides in length, which are at least 90 nucleotides in length, which are at least 100 nucleotides in length, which are at

least 200 nucleotides in length, which are at least 300 nucleotides in length, which are at least 400 nucleotides in length, which are at least 500 nucleotides in length, which are at least 600 nucleotides in length, or which preferably are full-length.

Another embodiment of the present invention is an isolated nucleic acid molecule selected from the group consisting of: (i) a nucleic acid molecule comprising (a) a nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO:58, or a fragment thereof of at least 55 nucleotides in length; (b) a nucleic acid linker of $(XXX)_n$ wherein n=0 to 60; and (c) a nucleic acid molecule comprising a nucleic acid sequence that is at least 87 percent identical to a nucleic acid sequence selected from the group consisting of SEQ ID NO:46 and SEQ ID NO:49, or a fragment thereof of at least 55 nucleotides in length, such that said nucleic acid molecule of (i) encodes a canine IL-12 single chain protein; and a nucleic acid molecule fully complementary to the coding strand of a nucleic acid molecule as set forth in (i). Preferred nucleic acid molecules include nucleic acid sequences that are at least 87%, at least 88%, at least 89%, more preferably at least 90%, even more preferably at least 95% identical to SEQ ID NO:52, SEQ ID NO:58, SEQ ID NO:46, and SEQ ID NO:49. Preferred fragment lengths include fragments of SEQ ID NO:52, SEQ ID NO:58, SEQ ID NO:46, and SEQ ID NO:49, which are at least 55 nucleotides in length, which are at least 60 nucleotides in length, which are at least about 65 nucleotides in length, which are at least 70 nucleotides in length, which are at least 80 nucleotides in length, which are at least 90 nucleotides in length, which are at least 100 nucleotides in length, which are at least 200 nucleotides in length, which are at least 300 nucleotides in length, which are at

least 400 nucleotides in length, which are at least 500 nucleotides in length, which are at least 600 nucleotides in length, or which preferably are full-length.

Preferred portions, or fragments, of a feline IL-18, feline caspase-1, canine or feline IL-12 single chain protein of the present invention include at least 15 amino acids, at least 20 amino acids, at least 25 amino acids, at least 30 amino acids, at least 35 amino acids, at least 40 amino acids, at least 45 amino acids, at least 50 amino acids, at least 60 amino acids, at least 75 amino acids or at least 100 amino acids. An IL-18 or IL-12 single chain protein of the present invention can include at least a portion of an IL-18 or IL-12 single chain protein that is capable of binding to an IL-18 or IL-12 receptor, respectively. These receptors are known to those of skill in the art, and are described in Janeway et al., in *Immunobiology, the Immune System in Health and Disease*, Garland Publishing, Inc., NY, 1996 (which is incorporated herein by this reference in its entirety). The IL-18 or IL-12 receptor-binding portion of an IL-18 or IL-12 protein, respectively, can be determined by incubating the protein with an isolated IL-18 or IL-12 receptor, as appropriate, or a cell having an IL-18 or IL-12 receptor on its surface, as appropriate. IL-18 or IL-12 protein binding to purified IL-18 or IL-12 receptor, respectively, can be determined using methods known in the art including Biacore® screening, confocal immunofluorescent microscopy, immunoprecipitation, gel chromatography, determination of inhibition of binding of antibodies that bind specifically to the IL-18 or IL-12 binding domain of an IL-18 or IL-12 receptor, ELISA using an IL-18 or IL-12 receptor, respectively, labeled with a detectable tag such as an enzyme or chemiluminescent tag or yeast-2 hybrid technology. A caspase-1 protein of the present invention can include at least a portion of a caspase-1 protein that is capable cleaving pro-IL-18 to mature IL-18. The ability of the caspase-1

protein to cleave IL-18 can be determined by methods known in the art, including methods such as Biacore® screening, confocal immunofluorescent microscopy, immunoprecipitation, gel chromatography, determination of inhibition of cleavage upon binding of antibodies that bind specifically to either IL-18 or caspase-1, and enzymatic assays.

The present invention also includes mimetopes of feline IL-18, feline caspase-1, and canine and/or feline IL-12 single chain proteins of the present invention. As used herein, a mimetope of an immunoregulatory protein of the present invention refers to any compound that is able to mimic the activity of such a feline IL-18, feline caspase-1, and canine and/or feline IL-12 single chain protein, respectively, often because the mimetope has a structure that mimics the particular protein. Mimetopes can be, but are not limited to: peptides that have been modified to decrease their susceptibility to degradation such as all-D retro peptides; anti-idiotypic and/or catalytic antibodies, or fragments thereof; non-proteinaceous immunogenic portions of an isolated protein (e.g., carbohydrate structures); and/or synthetic or natural organic molecules, including nucleic acids. Such mimetopes can be designed using computer-generated structures of proteins of the present invention. Mimetopes can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

Furthermore, it is known in the art that there are commercially available computer programs for determining the degree of similarity between two nucleic acid or protein sequences. These computer programs include various known methods to determine the percentage identity and the number and length of gaps between hybrid nucleic acid

molecules or proteins. Preferred methods to determine the percent identity among amino acid sequences and also among nucleic acid sequences include analysis using one or more of the commercially available computer programs designed to compare and analyze nucleic acid or amino acid sequences. These computer programs include, but are not limited to, the SeqLab® Wisconsin Package™ Version 10.0-UNIX sequence analysis software, available from Genetics Computer Group, Madison, WI; and DNAsis® sequence analysis software, version 2.0, available from Hitachi Software, San Bruno, CA. Such software programs represent a collection of algorithms paired with a graphical user interface for using the algorithms. The DNAsis version 2.0 software and SeqLab Wisconsin Package Version 10.0-UNIX software, for example, employ a particular algorithm, the Needleman-Wunsch algorithm to perform pair-wise comparisons between two sequences to yield a percentage identity score, see Needleman, S.B. and Wunsch, C.D., 1970, *J. Mol. Biol.*, 48, 443, which is incorporated herein by reference in its entirety. Such algorithms, including the Needleman-Wunsch algorithm, are commonly used by those skilled in the nucleic acid and amino acid sequencing art to compare sequences. A preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Needleman-Wunsch algorithm, available in the SeqLab Wisconsin Package Version 10.0-UNIX software (hereinafter "SeqLab"), using the Pairwise Comparison/Gap function with the nwsgapdna.cmp scoring matrix, the gap creation penalty and the gap extension penalties set at default values, and the gap shift limits set at maximum (hereinafter referred to as "SeqLab default parameters"). An additional preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes

using the Higgins-Sharp algorithm, available in the DNAsis version 2.0 software (hereinafter "DNAsis"), with the gap penalty set at 5, the number of top diagonals set at 5, the fixed gap penalty set at 10, the k-tuple set at 2, the window size set at 5, and the floating gap penalty set at 10. A particularly preferred method to determine percent identity among amino acid sequences and also among nucleic acid sequences includes using the Needleman-Wunsch algorithm available in the DNAsis version 2.0 software, using the GCG default parameter function.

Another embodiment of the present invention includes a nucleic acid molecule that is selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:66, and SEQ ID NO:68, and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule comprising any of said nucleic acid sequences. An allelic variant of a feline and/or canine nucleic acid molecule of the present invention, including the particular SEQ ID NO's cited herein, is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including the particular SEQ ID NO's cited herein, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Also included in the term allelic variant are allelic variants of cDNAs derived from such genes. Because natural selection typically selects against alterations that affect function, allelic variants usually encode

proteins having similar activity to that of the protein encoded by the gene to which they are being compared. Allelic variants of genes or nucleic acid molecules can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to be found within a given animal, since the respective genomes are diploid, and sexual reproduction will result in the reassortment of alleles. As such, a nucleic acid molecule of the present invention can be an allelic variant that includes a similar but not identical sequence to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:9, SEQ ID NO:41, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:66, and SEQ ID NO:68, and/or any other nucleic acid molecule cited herein.

In another embodiment of the present invention, a nucleic acid molecule of the invention is selected from the group consisting of (a) a nucleic acid molecule comprising a nucleic acid sequence that encodes a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:62, and SEQ ID NO:67, and (b) a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said amino acid sequences of (a).

Another embodiment of the present invention includes feline IL-18 nucleic acid molecules of the present invention, wherein said nucleic acid molecules encode a protein having a function selected from the group consisting of (i) eliciting an immune response against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12; (ii) selectively binding to an antibody raised against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and (iii) exhibiting IL-18 activity. Methods to elicit an immune response and to determine whether an antibody can selectively bind to a particular protein or antigen are known in the art, see, for example, Harlow, et al. (1988) *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow, et al. is incorporated by reference herein in its entirety. Methods to determine whether an IL-18 protein has IL-18 activity are known in the art, and include determining whether IL-18 has the activity of stimulating T cells to produce interferon gamma (IFN-γ).

Another embodiment of the present invention includes caspase-1 nucleic acid molecules of the present invention that encode a protein having a function selected from the group consisting of (i) eliciting an immune response against a caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, (ii) selectively binding to an antibody raised against caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and (iii) exhibiting caspase-1 activity. Methods to elicit an immune response and to determine whether an antibody can selectively bind to a particular protein or antigen

are known in the art, see, for example, Harlow, et al. (1988) *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow, et al. is incorporated by reference herein in its entirety. Methods to determine whether a caspase-1 protein has caspase-1 activity are known in the art, and include, for example, determining if the caspase-1 protein has the ability to cleave the precursor form of IL-18 resulting in a biologically active IL-18.

Another embodiment of the present invention includes canine and feline IL-12 single chain nucleic acid molecules of the present invention, wherein a said nucleic acid molecule encodes a protein having a function selected from the group consisting of (i) eliciting an immune response against an IL-12 protein having an amino acid selected from the group consisting of SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:62, and SEQ ID NO:67, (ii) selectively binding to an antibody raised against an IL-12 protein having an amino acid sequence selected from the group consisting from the group of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67, and (iii) exhibiting IL-12 activity. Methods to elicit an immune response and to determine whether an antibody can selectively bind to a particular protein or antigen are known in the art, see, for example, Harlow, et al. (1988) *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow, et al. is incorporated by reference herein in its entirety. Methods to determine whether an IL-12 protein has IL-12 activity are known in the art, and include determining if IL-12 has the activity of stimulating T cells to produce interferon gamma (IFN-γ).

A preferred nucleic acid molecule of the present invention includes a nucleic acid molecule selected from the group consisting of nFeIL-12p40-N_{985}, nFeIL-12p40_{987}, nFeIL-12p40_{921}, nFeIL-12p35_{666}, nFeIL-12p35-N_{561}, nFeIL-12p35_{591}, nCaIL-12p35_{666}, nCaIL-12p35_{1455}, nCaIL-12p35_{591}, nCaIL-12p40_{2267}, nCaIL-12p40_{921}, nCaIL-12p40_{98}, nFeIL-12_{1599}, nFeIL-12_{1533}, nCaIL-12_{1599}, and nCaIL-12_{1533}.

Another embodiment of the present invention includes an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence encoding an IL-18 protein selected from the group consisting of: a protein selected from the group consisting of (a) a protein having an amino acid sequence that is at least 92 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and (b) a protein comprising a fragment of a protein of (a), wherein said fragment is at least 30 amino acids in length; and a protein comprising at least 25 contiguous amino acids identical in sequence to at least 25 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12. Preferred IL-18 proteins include proteins that are at least about 90 percent identical, preferably at least about 92 percent identical, preferably at least about 94 percent identical, preferably at least about 96 percent identical, and even more preferably at least about 98 percent identical to SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12 or fragments thereof. Preferred fragments of IL-18 proteins include fragments of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12 that are at least about 20 amino acids in length, at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, preferably at least about 75 amino

acids in length, preferably at least about 100 amino acids in length, and more preferably are full-length. Preferred IL-18 proteins also include proteins that comprise at least 15 contiguous amino acids identical in sequence to at least 15 contiguous amino acids; at least 20 contiguous amino acids identical in sequence to at least 20 contiguous amino acids, preferably about 30 contiguous amino acids identical in sequence to at least 30 contiguous amino acids, preferably about 50 contiguous amino acids identical in sequence to at least 50 contiguous amino acids, preferably about 75 contiguous amino acids identical in sequence to at least 75 contiguous amino acids, preferably about 100 contiguous amino acids identical in sequence to at least 100 contiguous amino acids, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12.

Another embodiment of the present invention includes an isolated nucleic acid molecule selected from the group consisting of: a nucleic acid molecule having a nucleic acid sequence encoding caspase protein selected from the group consisting of: a protein selected from the group consisting of (a) a protein having an amino acid sequence that is at least 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and (b) a protein comprising a fragment of a protein of (a), wherein said fragment is at least 30 amino acids in length; and a protein comprising at least 25 contiguous amino acids identical in sequence to at least 25 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12. Preferred caspase-1 proteins include proteins that are at least about 85 percent identical,

at least about 87 percent identical, preferably at least about 90 percent identical, preferably at least about 93 percent identical, more preferably at least about 95 percent identical, and even more preferably about 98 percent identical to SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24 or fragments thereof. Preferred fragments of caspase-1 proteins include fragments of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24 include fragments that are at least about 20 amino acids in length, at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, preferably at least about 75 amino acids in length, preferably at least about 100 amino acids in length, and more preferably are full-length. Preferred caspase-1 proteins also include proteins that comprise at least 25 contiguous amino acids identical in sequence to at least 25 contiguous amino acids; at least 20 contiguous amino acids identical in sequence to at least 20 contiguous amino acids, preferably about 30 contiguous amino acids identical in sequence to at least 30 contiguous amino acids, preferably about 50 contiguous amino acids identical in sequence to at least 50 contiguous amino acids, preferably about 75 contiguous amino acids identical in sequence to at least 75 contiguous amino acids, preferably about 100 contiguous amino acids identical in sequence to at least 100 contiguous amino acids, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24.

Another embodiment of the present invention includes a nucleic acid molecule having a nucleic acid sequence encoding an IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to a IL-12 p35 subunit domain, wherein said p40 subunit

domain is selected from the group consisting of: (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p40 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30, and wherein said p35 domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p35 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36. Preferred p40 subunit proteins and/or p35 subunit proteins include proteins that are at least about 84 percent identical, preferably at least about 87 percent identical, preferably at least about 90 percent identical, and even more preferably at least about 95 percent identical to SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36 or fragments thereof. Preferred fragments of IL-12 single chain proteins include fragments of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36 include fragments that are at least about 30 amino acids in length, at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, preferably at least about 75 amino acids in length, preferably at least about 100

amino acids in length, and more preferably are full-length. Preferred IL-12 single chain proteins also include proteins that comprise at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids, preferably about 30 contiguous amino acids identical in sequence to at least 30 contiguous amino acids, preferably about 50 contiguous amino acids identical in sequence to at least 50 contiguous amino acids, preferably about 75 contiguous amino acids identical in sequence to at least 75 contiguous amino acids, preferably about 100 contiguous amino acids identical in sequence to at least 100 contiguous amino acids, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36.

Another embodiment of the present invention includes a nucleic acid molecule having a nucleic acid sequence encoding an IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to a IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of: (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p40 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, and wherein said p35 domain is selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50, (ii) a

p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p35 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50. Preferred p40 subunit proteins and/or p35 subunit proteins include proteins that are at least about 84 percent identical, preferably at least about 87 percent identical, preferably at least about 90 percent identical, and even more preferably at least about 95 percent identical to SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59 or fragments thereof. Preferred fragments of IL-12 single chain proteins include fragments of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, and SEQ ID NO:36 include fragments that are at least about 40 amino acids in length, at least about 50 amino acids in length, at least about 60 amino acids in length, at least about 70 amino acids in length, preferably at least about 80 amino acids in length, preferably at least about 100 amino acids in length, and more preferably are full-length. Preferred IL-12 single chain proteins also include proteins that comprise at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids, preferably about 35 contiguous amino acids identical in sequence to at least 35 contiguous amino acids, preferably about 50 contiguous amino acids identical in sequence to at least 50 contiguous amino acids, preferably about 75 contiguous amino acids identical in sequence to at least 75 contiguous amino acids, preferably about 100 contiguous amino acids identical in sequence to at least 100 contiguous amino acids, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59.

Another embodiment of the present invention includes a nucleic acid molecule comprising a nucleic acid sequence fully complementary to the coding strand of any of the nucleic acid molecules of the present invention. Another embodiment of the present invention includes a nucleic acid molecule that comprises a nucleic acid sequence that encodes a protein selected from the group consisting of an IL-18 protein, a caspase-1 protein, and an IL-12 single chain protein.

Another embodiment of the present invention includes a nucleic acid molecule that is selected from the group consisting of a nucleic acid molecule comprising a nucleic acid sequence encoding a protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO;18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:62, and SEQ ID NO:67; and a nucleic acid molecule comprising an allelic variant of a nucleic acid molecule encoding a protein having any of said nucleic acid molecules set forth in this paragraph. In another embodiment, a nucleic acid molecule encoding an IL-12 single chain protein of the present invention further comprises a nucleic acid molecule encoding a linker.

The present invention also includes oligonucleotides, recombinant molecules, recombinant viruses and recombinant cells comprising such nucleic acid molecules and methods to produce such nucleic acid molecules, oligonucleotides, recombinant molecules, recombinant viruses and recombinant cells.

Knowing the nucleic acid sequences of certain nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of

such nucleic acid molecules, e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions, and (c) obtain other nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. A preferred library to screen or from which to amplify nucleic acid molecules is a feline or canine mast library or a feline or canine peripheral blood mononuclear cell library. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid.*

Oligonucleotides of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such oligonucleotides is the size required for formation of a stable hybrid between an oligonucleotide and a complementary sequence on a larger nucleic acid molecule of the present invention, typically from about 12 to 15 to about 17 to 18 nucleotides depending on the GC/AT content. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit protein production or activity, e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents. The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

One embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention, inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used in the cloning, sequencing, and/or otherwise manipulating of the nucleic acid molecules of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression vector. The phrase operatively linked refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the nucleic acid molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. Expression vectors can be either prokaryotic or eukaryotic, and are typically viruses or plasmids. Expression vectors of the present invention include any vectors that function, i.e., direct gene expression, in recombinant cells of the present invention, including in bacterial, fungal, insect, other animal, and plant cells. Preferred expression vectors of the present invention can direct gene

expression in bacterial, yeast, insect and mammalian cells, and more preferably in the cell types disclosed herein.

In particular, expression vectors of the present invention contain regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of nucleic acid molecules of the present invention. In particular, recombinant molecules of the present invention include transcription control sequences. Transcription control sequences are sequences that control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those that control transcription initiation, such as promoter, enhancer, operator and repressor sequences. Suitable transcription control sequences include any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, or insect and mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, oxy-pro, omp/lpp, rrnB, bacteriophage lambda, such as lambda p_L and lambda p_R and fusions that include such promoters, bacteriophage T7, T7*lac*, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoter, antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus, such as immediate early promoter, simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate and nitrate transcription control sequences as well as other

sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters, e.g., promoters inducible by interferons or interleukins.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transfer cells are disclosed herein. A recombinant cell is preferably produced by transforming a host cell with one or more recombinant molecules, each comprising one or more nucleic acid molecules of the present invention operatively linked to an expression vector containing one or more transcription control sequences, examples of which are disclosed herein.

Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ or a multicellular organism. It is to be noted that a cell line refers to any recombinant cell of the present invention that is not a transgenic animal. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed, i.e., recombinant, cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which

to transform a cell include nFeIL-18-N_{514}, nFeIL-18-C_{502}, nFeIL-18_{607}, nFeIL-18_{576}, nFeIL-18_{471}, nFeCasp-1_{1233}, nFeCasp-1-N_{526}, nFeCasp-1-C_{500}, nFeCasp-1_{1230}, nFeIL-12p40-N_{985}, nFeIL-$12p40_{987}$, nFeIL-$12p40_{921}$, nFeIL-$12p35_{666}$, nFeIL-12p35-N_{561}, nFeIL-$12p35_{591}$, nCaIL-$12p35_{666}$, nCaIL-$12p35_{1455}$, nCaIL-$12p35_{591}$, nCaIL-$12p40_{2267}$, nCaIL-$12p40_{921}$, and nCaIL-$12p40_{987}$, nCaIL-12_{1599}, nCaIL-12_{1533}, nFeIL-12_{1599}, and nFeIL-12_{1533}.

Recombinant molecules of the present invention may also (a) contain secretory signals, i.e., signal segment nucleic acid sequences, to enable an expressed protein of the present invention to be secreted from the cell that produces the protein and/or (b) contain fusion sequences which lead to the expression of nucleic acid molecules of the present invention as fusion proteins. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteosome, such as a ubiquitin fusion segment. Eukaryotic recombinant molecules may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequences of nucleic acid molecules of the present invention.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule, e.g., nucleic acid molecules encoding one or more proteins of the present invention and/or

other proteins. Host cells of the present invention either can be endogenously, i.e., naturally, capable of producing proteins of the present invention or can be capable of producing such proteins after being transformed with at least one nucleic acid molecule of the present invention. Host cells of the present invention can be any cell capable of producing at least one protein of the present invention, and include bacterial, fungal, including yeast, insect, and other animal and plant cells. Preferred host cells include bacterial, mycobacterial, yeast, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Pichia*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby canine kidney cell line), CRFK cells (Crandell feline kidney cell line), CV-1 cells (African monkey kidney cell line used, for example, to culture raccoon poxvirus), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1 $_{\chi}$3987 and SR-11 $_{\chi}$4072; *Pichia; Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; CV-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines, e.g., human, murine or chicken embryo fibroblast cell lines, myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK^{31} cells and/or HeLa cells.

Recombinant cells of the present invention can also be co-transformed with one or more recombinant molecules including IL-18, caspase-1, IL-12 single chain nucleic acid molecules encoding one or more proteins of the present invention and one or more other nucleic acid molecules encoding other compounds.

Recombinant DNA technologies can be used to improve expression of transformed nucleic acid molecules by manipulating, for example, the number of copies of the nucleic acid molecules within a host cell, the efficiency with which those nucleic acid molecules are transcribed, the efficiency with which the resultant transcripts are translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of nucleic acid molecules of the present invention include, but are not limited to, operatively linking nucleic acid molecules to high-copy number plasmids, integration of the nucleic acid molecules into one or more host cell chromosomes, addition of vector stability sequences to plasmids, substitutions or modifications of transcription control signals, e.g., promoters, operators, enhancers, substitutions or modifications of translational control signals, e.g., ribosome binding sites, Shine-Dalgarno sequences, modification of nucleic acid molecules of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing nucleic acid molecules encoding such a protein.

Isolated feline IL-18, feline caspase-1, feline and/or canine IL-12 single chain proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred

cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a protein of the present invention. Such a medium typically comprises an aqueous medium having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

Depending on the vector and host system used for production, resultant proteins of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane. The phrase recovering the protein, as well as similar phrases, refers to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in

substantially pure form. As used herein, substantially pure refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity.

The present invention also includes isolated, i.e., removed from their natural milieu, antibodies that selectively bind to proteins of the present invention or a mimetope thereof, e.g., anti-feline IL-18, feline caspase-1, feline and canine IL-12 single antibodies. As used herein, the term selectively binds to a protein refers to the ability of antibodies of the present invention to preferentially bind to specified proteins and mimetopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays, e.g., ELISA, immunoblot assays, etc.; see, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated herein by reference in its entirety. For example, an anti-feline IL-18 antibody of the present invention preferably selectively binds to a feline IL-18 protein in such a way as to inhibit the function of that protein.

The antibodies of the present invention bind to the proteins of the present invention, but not to similar proteins of other species. For instance, the antibodies that specifically bind feline IL-18 do not bind canine IL-18.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, or can be functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to one or more epitopes.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effective amount of a protein, peptide or mimetope of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) to evaluate the immune status in felids and canids with diseases such as allergy, cancer and pathogen infections. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to cells. Targeting can be accomplished by conjugating, i.e., stably joining, such antibodies to the cytotoxic agents using techniques known to those skilled in the art. Suitable cytotoxic agents are known to those skilled in the art. Furthermore, antibodies of the present invention can be used to detect for example, feline IL-18, caspase-1, canine IL-12 single chain, and/or feline IL-12 single chain in a putative IL-18, caspase-1, canine IL-12 single chain, and/or feline IL-12 single chain containing biological sample, by contacting the putative IL-18, caspase-1, canine IL-12 single chain, and/or feline IL-12 single chain containing biological sample with the appropriate anti-IL-18, caspase-1, canine IL-12 single chain, and/or feline IL-12 single chain antibodies under conditions suitable for formation of an antigen-antibody complex, and then detecting said

complex. Methods to detect said method are known to those skilled in the art and are contained herein.

The present invention includes proteins comprising SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:12, SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, SEQ ID NO:59, SEQ ID NO:62, and SEQ ID NO:67 as well as nucleic acid molecules encoding such proteins.

Preferred feline IL-18 proteins of the present invention include PFeIL-18-$N_{133,}$ PFeIL-18-$C_{154,}$ PFeIL-$18_{192,}$ and/or PFeIL-18_{157}. In one embodiment, a preferred feline IL-18 protein of the present invention has an amino acid sequence that includes SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and/or SEQ ID NO:12 and is preferably encoded by a nucleic acid molecule having nucleic acid sequences SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:9 and/or SEQ ID NO:11. Such proteins are preferably encoded by a nucleic acid molecule comprising nFeIL-18-N_{514}, nFeIL-18-$C_{502,}$ nFeIL-18_{607}, nFeIL-$18_{576,}$ and/or nFeIL-18_{471}.

Preferred feline caspase-1 proteins of the present invention include proteins encoded by a nucleic acid molecule comprising nFeCasp-1_{1233}, nFeCasp-1-$N_{526,}$ nFeCasp-1-$C_{500,}$ and/or nFeCasp-1_{1230}. Preferred feline caspase-1 proteins are PFeCasp-1_{410}, PFeCasp-1-N_{169}, and/or PFeCasp-1-$C_{120.}$ In one embodiment, a preferred feline caspase-1 protein of the present invention is encoded by SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:20, and/or SEQ ID NO:23, and, as such, has an amino acid sequence that includes SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21 and/or SEQ ID NO:24.

Preferred canine and feline IL-12 proteins of the present invention include proteins encoded by a nucleic acid molecule comprising nFeIL-12_{1599}, nFeIL-12_{1533}, nCaIL-12_{1599}, and/or nCaIL-12_{1533}. Preferred feline and canine IL-12 proteins are nFeIL-12p40-N_{985}, nFeIL-12p40_{987}, nFeIL-12p40_{921}, nFeIL-12p35_{666}, nFeIL-12p35-N_{561}, nFeIL-12p35_{591}, nCaIL-12p35_{666}, nCaIL-12p35_{1455}, nCaIL-12p35_{591}, nCaIL-12p40_{2267}, nCaIL-12p40_{921}, and nCaIL-12p40_{987}. In one embodiment, a preferred canine and feline IL-12 single chain protein of the present invention is encoded by SEQ ID NO:38, SEQ ID NO:43, SEQ ID NO:61, and/or SEQ ID NO:66, and, as such, has an amino acid sequence that includes SEQ ID NO:39, SEQ ID NO:44, SEQ ID NO:62, and/or SEQ ID NO:67.

More preferred canine and feline IL-12 single chain proteins of the present invention include proteins encoded by a nucleic acid molecule comprising nFeIL-12p40-N_{985}, nFeIL-12p40_{987}, nFeIL-12p40_{921}, nFeIL-12p35_{666}, nFeIL-12p35-N_{561}, nFeIL-12p35_{591}, nCaIL-12p35_{666}, nCaIL-12p35_{1455}, nCaIL-12p35_{591}, nCaIL-12p40_{2267}, nCaIL-12p40_{921}, nCaIL-12p40_{987}, nCaIL-12_{1533}, nCaIL-12_{1599}, nFeIL-12_{1533}, and nFeIL-12_{1599}. Preferred feline and canine IL-12 single chain proteins comprise PFeIL-12p40-N_{328}, PFeIL-12p40_{329}, PFeIL-12p40_{307}, PFeIL-12p35_{222}, PFeIL-12p35-N_{187}, PFeIL-12p35_{197}, PCaIL-12p35_{222}, PCaIL-12p35_{197}, PCaIL-12p40_{307}, PCaIL-12p40_{329}, PFeIL-12_{533}, PFeIL-12_{511}, PCaIL-12_{533}, and PCaIL-12_{511}. In one embodiment, a preferred canine and feline IL-12 single chain protein of the present invention is encoded by a nucleic acid comprising SEQ ID NO:28, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:35, SEQ ID NO:46, SEQ ID NO:49, SEQ ID NO:52, SEQ ID NO:55, SEQ ID NO:58, SEQ ID NO:101, SEQ ID NO:104, SEQ ID NO:107, SEQ ID NO:38, SEQ ID NO:43, SEQ ID NO:61, SEQ ID NO:66, and, as such, has an amino acid sequence that includes SEQ ID

NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67.

As used herein, an isolated protein of the present invention can be a full-length protein or any homolog of such a protein. An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to bind to a receptor or a protein. Examples of protein homologs of the present invention include proteins of the present invention in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation and/or addition of glycerophosphatidyl inositol) such that the protein homolog includes at least one epitope capable of eliciting an immune response against the parent protein, of binding to an antibody directed against the parent protein and/or of binding to the parent's receptor, where the term parent refers to the longer and/or full-length protein that the homolog is derived from. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of an immunoregulatory protein of the present invention, depending upon which protein is administered to an animal. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art.

Homologs of proteins of the present invention can be the result of natural allelic variation, including natural mutation. Protein homologs of the present invention can also

be produced using techniques known in the art including, but not limited to, direct modifications to the protein and/or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

One embodiment of the present invention is an IL-18 protein selected from the group consisting of: (i) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12; and (ii) a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8 and SEQ ID NO:12. Another embodiment is a caspase-1 protein selected from the group consisting of: (i) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24; and (ii) a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24. Yet another embodiment is a feline IL-12 single chain protein selected from the group consisting of: (i) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:38, and SEQ ID NO:44; and (ii) a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein selected from the group consisting of SEQ ID NO:38, and SEQ ID NO:44; or a canine Il-12 single chain protein selected from the group consisting of: (i) a protein having an amino acid sequence selected from the group consisting of SEQ ID NO:62 and SEQ ID NO:67; and (ii) a protein encoded by an allelic variant of a nucleic acid molecule

encoding a protein selected from the group consisting of SEQ ID NO:62 and SEQ ID NO:67.

One embodiment of the present invention includes an isolated IL-18 protein selected from the group consisting of (i) an isolated protein of at least 25 amino acids in length, wherein said protein has an at least 25 contiguous amino acid region identical in sequence to a 25 contiguous amino acid region selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12; and (ii) an isolated protein having an amino acid sequence that is at least 92 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and a fragment thereof of at least 30 nucleotides. Preferred proteins have an at least 15 contiguous amino acid region identical with a 15 contiguous amino acid region, an at least 20 contiguous amino acid region identical with a 20 contiguous amino acid region, an at least 30 contiguous amino acid region identical with a 30 contiguous amino acid region, an at least 40 contiguous amino acid region identical with a 40 contiguous amino acid region, an at least 50 contiguous amino acid region contiguous with a 50 contiguous amino acid region, an at least 75 contiguous amino acid region contiguous with a 75 contiguous amino acid region, preferably an at least 100 contiguous amino acid region contiguous with a 100 contiguous amino acid region, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12. In another embodiment, preferred proteins have an amino acid sequence that is at least 90 percent identical, at least 92 percent identical, preferably at least 94 percent identical, preferably at least 96 percent identical, and even

more preferably at least about 98 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and a fragment thereof of at least 20 amino acids, at least 30 amino acids, at least 50 amino acids, at least 75 amino acids, preferably at least 100 amino acids, and more preferably a full-length protein.

In a preferred embodiment, IL-18 proteins of the present invention has a function selected from the group consisting of: (i) eliciting an immune response against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, (ii) selectively binding to an antibody raised against an IL-18 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, and SEQ ID NO:12, and (iii) exhibiting IL-18 activity.

One embodiment of the present invention includes an isolated caspase-1 protein selected from the group consisting of (i) an isolated protein of at least 25 amino acids in length, wherein said protein has an at least 25 contiguous amino acid region identical in sequence to a 25 contiguous amino acid region selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24; and (ii) an isolated protein having an amino acid sequence that is at least 85 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and a fragment thereof of at least 30 nucleotides. Preferred proteins have an at least 25 contiguous amino acid region identical with a 25 contiguous amino acid region, an at least 20 contiguous amino acid region identical with a 20 contiguous amino acid region, an at least 30 contiguous amino acid region identical

with a 30 contiguous amino acid region, an at least 40 contiguous amino acid region identical with a 40 contiguous amino acid region, an at least 50 contiguous amino acid region contiguous with a 50 contiguous amino acid region, an at least 75 contiguous amino acid region contiguous with a 75 contiguous amino acid region, preferably an at least 100 contiguous amino acid region contiguous with a 100 contiguous amino acid region, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24. In another embodiment, preferred proteins have an amino acid sequence that is at least 85 percent identical, at least 88 percent identical, preferably at least 90 percent identical, and more preferably at least about 95 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and a fragment thereof of at least 30 amino acids, at least 50 amino acids, at least 75 amino acids, preferably at least 100 amino acids, and more preferably a full-length protein.

In a preferred embodiment, a caspase protein of the present invention has a function selected from the group consisting of: (i) eliciting an immune response against a caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, (ii) selectively binding to an antibody raised against a caspase-1 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:18, SEQ ID NO:21, and SEQ ID NO:24, and (iii) exhibiting caspase-1 activity.

One embodiment of the present invention includes an isolated IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to an IL-12 p35 subunit domain,

wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p40 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:27 and SEQ ID NO:30. The p35 subunit is preferably selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 30 amino acids in length, and (iii) a p35 subunit protein comprising at least 23 contiguous amino acids identical in sequence to at least 23 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:33 and SEQ ID NO:36. Preferred amino acid sequences have an at least 23 contiguous amino acid region identical with a 23 contiguous amino acid region, an at least 30 contiguous amino acid region identical with a 30 contiguous amino acid region, an at least 40 contiguous amino acid region identical with a 40 contiguous amino acid region, an at least 50 contiguous amino acid region contiguous with a 50 contiguous amino acid region, an at least 75 contiguous amino acid region contiguous with a 75 contiguous amino acid region, preferably an at least 100 contiguous amino acid region contiguous with a 100 contiguous amino acid region, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected

from the group consisting of SEQ ID NO:27 and SEQ ID NO:30 In another embodiment, preferred proteins have an amino acid sequence that is at least 84 percent identical, at least 86 percent identical, at least 88 percent identical, preferably at least 90 percent identical, and more preferably at least about 95 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, and a fragment thereof of at least 30 amino acids, at least 50 amino acids, at least 75 amino acids, preferably at least 100 amino acids, and more preferably a full-length protein.

In a preferred embodiment, an IL-12 single chain protein of the present invention has a function selected from the group consisting of: (i) eliciting an immune response against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67; (ii) selectively binding to an antibody raised against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67; and (iii) exhibiting IL-12 activity.

One embodiment of the present invention includes an isolated IL-12 single chain protein comprising an IL-12 p40 subunit domain linked to an IL-12 p35 subunit domain, wherein said p40 subunit domain is selected from the group consisting of (i) a p40 subunit protein having an amino acid sequence that is at least 84 percent identical to an

amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59, (ii) a p40 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p40 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:53 and SEQ ID NO:59. The p35 subunit is preferably selected from the group consisting of (i) a p35 subunit protein having an amino acid sequence that is at least 84 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50, (ii) a p35 subunit protein comprising a fragment of a protein of (i), wherein said fragment is at least 40 amino acids in length, and (iii) a p35 subunit protein comprising at least 31 contiguous amino acids identical in sequence to at least 31 contiguous amino acids of an amino acid sequence selected from the group consisting of SEQ ID NO:47 and SEQ ID NO:50. Preferred amino acid sequences have an at least 23 contiguous amino acid region identical with a 23 contiguous amino acid region, an at least 30 contiguous amino acid region identical with a 30 contiguous amino acid region, an at least 40 contiguous amino acid region identical with a 40 contiguous amino acid region, an at least 50 contiguous amino acid region contiguous with a 50 contiguous amino acid region, an at least 75 contiguous amino acid region contiguous with a 75 contiguous amino acid region, preferably an at least 100 contiguous amino acid region contiguous with a 100 contiguous amino acid region, and most preferably a full-length protein identical in sequence to a full-length protein of an amino acid sequence selected from the group consisting of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59. In another embodiment, preferred proteins have an amino acid sequence that

is at least 84 percent identical, at least 86 percent identical, at least 88 percent identical, preferably at least 90 percent identical, and more preferably at least about 95 percent identical to an amino acid sequence selected from the group consisting of SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, and a fragment thereof of at least 30 amino acids, at least 50 amino acids, at least 75 amino acids, preferably at least 100 amino acids, and more preferably a full-length protein.

In a preferred embodiment, an IL-12 single chain protein of the present invention has a function selected from the group consisting of: (i) eliciting an immune response against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:62 and SEQ ID NO:67, (ii) selectively binding to an antibody raised against an IL-12 protein having an amino acid sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:30, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:47, SEQ ID NO:50, SEQ ID NO:53, and SEQ ID NO:59, SEQ ID NO:102, SEQ ID NO:105, SEQ ID NO:108, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:62, and/or SEQ ID NO:67, and (iii) exhibiting IL-12 activity.

One embodiment of the present invention is a therapeutic composition that, when administered to a animal in an effective manner, is capable of protecting that animal from a disease such as, for example, allergy, cancer or inflammation. Therapeutic compositions of the present invention include protective compounds that are capable of regulating feline IL-18, feline caspase-1, or feline or canine IL-12 protein amounts and/or activity. A protective compound of the present invention is capable of regulating feline IL-18, feline caspase-1, or feline or canine IL-12 activity and/or availability. Examples of protective compounds related to feline and canine proteins of the present invention

include an isolated antibody that selectively binds to either feline IL-18, feline caspase-1, or feline or canine IL-12 or other inhibitors or activators of feline IL-18, feline caspase-1, or feline or canine IL-12 activity or amount. Other examples of protective compounds include an isolated nucleic acid molecule of the present invention; an isolated protein of the present invention; a mimetope of a protein of the present invention, a multimeric form of any of said proteins, or an inhibitor identified by its ability to inhibit the activity of any of said proteins; such an inhibitor can inhibit binding of the respective protein with its receptor, or inhibit the activity of the respective protein. Methods to perform such assays to measure binding and/or activity of protein of the present invention are known to those of skill in the art, and are described, for example, in Janeway et al., *ibid.* As such, these protective compounds may include antibodies, peptides, substrate analogs, and other large or small molecules which can be organic or inorganic. As used herein, a protective compound refers to a compound, that when administered to an animal in an effective manner, is able to treat, ameliorate, and/or prevent a disease due to allergy, cancer or infection. Examples of proteins, nucleic acid molecules, antibodies and/or inhibitors of the present invention are disclosed herein.

The present invention also includes a therapeutic composition comprising at least one compound of the present invention in combination with at least one additional therapeutic compound. Examples of such compounds are disclosed herein.

The efficacy of a therapeutic composition of the present invention to protect an animal from a disease mediated by feline IL-18, feline caspase-1, or feline or canine IL-12 can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present

invention), detection of the amount of feline IL-18, feline caspase-1, or feline or canine IL-12,or detection of cellular immunity within the treated animal. Therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

Therapeutic compounds of the present invention can be administered to any animal susceptible to such therapy, preferably to mammals, and more preferably to dogs, cats, humans, ferrets, horses, cattle, sheep and/or other pets, economic food animals, and/or zoo animals. Preferred animals include dogs and cats.

A therapeutic composition of the present invention is administered to an animal in an effective manner such that the composition is capable of regulating an immune response in that animal. Therapeutic compositions of the present invention can be administered to animals prior to the onset of a disease (i.e. as a preventative vaccine) and/or can be administered to animals after onset of a disease in order to treat the disease (i.e. as a therapeutic vaccine). Preferred diseases to prevent and/or treat include autoimmune diseases, allergic reactions, infectious diseases, tumor development, inflammatory diseases and/or graft rejection. In one embodiment, a therapeutic composition of the present invention is administered with an antigen to enhance an immune response against that antigen. Such administration can include, but is not limited to, oral, intravenous, intramuscular, intra ocular, mucosal, intranasal, subcutaneous, topical or transdermal application. In order to protect an animal from disease, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting that animal from a disease. Therapeutic compositions of the present invention can be administered to

animals prior to disease in order to prevent disease and/or can be administered to animals after disease occurs. The exact dose, administration regimen, and administration route of therapeutic compositions of the present invention can be determined by one skilled in the art. A suitable single dose is a dose that is capable of regulating the immune response in an animal when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimetope or antibody therapeutic composition is from about 1 microgram (μg) to about 10 milligrams (mg) of the therapeutic composition per kilogram body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 μg to about 1 mg of the therapeutic composition per kg body weight of the animal is administered from about one to about two times over a time period of from about 2 weeks to about 12 months.

A therapeutic composition of the present invention can include at least one of the following: excipient, an adjuvant and a carrier. Therapeutic compositions of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Nonaqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical

stability. Examples of buffers include phosphate buffer, bicarbonate buffer and Tris buffer, while examples of preservatives include thimerosal, or o-cresol, formalin and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

Therapeutic compositions of the present invention can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interferon gamma, transforming growth factor beta, RANTES (regulated upon activation, normal T cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, GA), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, MT); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants

of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

Therapeutic compositions of the present invention can include a carrier. Carriers include compounds that increase the half-life of a protective compound in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, other lipid or lipid containing formulations, including cationic lipids or lipid mixtures including cationic lipids, bacteria, viruses, other cells, oils, esters, and glycols.

A therapeutic composition can be a controlled release formulation that is capable of slowly releasing a protective compound of the present invention into an animal. As used herein, a controlled release formulation comprises a composition or protective compound of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, other lipids or lipid-containing formulations and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel *in situ*. Preferred controlled release formulations are biodegradable, i.e., bioerodible.

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition to regulate an immune response in an animal. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release

formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a therapeutic protein or therapeutic RNA (e.g. antisense RNA, ribozyme, triple helix forms or RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked (i.e. not packaged in a viral coat or cellular membrane) nucleic acid as a genetic vaccine (e.g. as naked DNA or RNA molecules, such is taught, for example, in Wolff et al., 1990, Science 247, p 1465-68) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e. the nucleic acid molecule is delivered by a viral or cellular vehicle).

One embodiment of a therapeutic composition of the present invention is a naked nucleic acid, a recombinant virus or a recombinant cell vaccine or therapy. Naked nucleic acid molecules of the present invention can be administered by a variety of methods. Suitable delivery methods include, for example, intramuscular injection, subcutaneous injection, intradermal injection, intradermal scarification, particle bombardment, oral application, topical application and nasal application, with intramuscular injection, intradermal injection, intradermal scarification and particle bombardment being preferred. A preferred single dose of a naked nucleic acid molecule ranges from about 1 nanogram (ng) to about 1 milligram (mg), depending on the route of administration and/or method

of delivery, as can be determined by those skilled in the art. Examples of administration methods are disclosed, for example, in U.S. Patent No. 5,204,253, by Bruner, et al., issued April 20, 1993, PCT Publication No. WO 95/19799, published July 27, 1995, by McCabe, and PCT Publication No. WO 95/05853, published March 2, 1995, by Carson, et al. Naked nucleic acid molecules of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) and/or with a carrier (e.g., lipid-based vehicles), or it can be bound to microparticles (e.g., gold particles).

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA, e.g., antisense RNA, ribozyme, triple helix form or RNA drug, in the animal. Nucleic acid molecules can be delivered to an animal in a variety of methods including, but not limited to, (a) administering a naked, i.e., not packaged in a viral coat or cellular membrane, nucleic acid as a genetic therapy or vaccine, e.g., as naked DNA or RNA molecules, such as is taught, for example in Wolff et al., 1990, *Science 247*, 1465-1468, or (b) administering a nucleic acid molecule packaged as a recombinant virus therapy or vaccine or as a recombinant cell therapy or vaccine, i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle.

A genetic, i.e., naked nucleic acid, therapy vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication or otherwise amplification, competent. A genetic therapy or vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention operatively linked to a transcriptional control sequence in the form of, for example, a dicistronic

recombinant molecule. Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and/or retroviruses, with those based on alphaviruses (such as sindbis or Semliki forest virus) species-specific herpesviruses and/or poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of a "strong" polyadenylation signal is also preferred.

Genetic therapies and vaccines of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal, topical and oral routes of administration being preferred. A preferred single dose of a genetic therapy or vaccine ranges from about 1 nanogram (ng) to about 600 μg, depending on the route of administration and/or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized and/or topically. Genetic therapies or vaccines of the present invention can be contained in an aqueous excipient, e.g., phosphate buffered saline, alone or in a carrier, e.g., lipid-based vehicles. One embodiment is a nucleic acid-lipid complex, preferably a nucleic acid-cationic lipid complex.

A recombinant virus therapy or vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule

is packaging- or replication-deficient and/or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses, such as Sindbis virus, raccoon poxviruses, species-specific herpesviruses and species-specific poxviruses. An example of methods to produce and use recombinant virus therapies and vaccines is disclosed in U.S. Patent No. 5,766,602, Xiong et al., issued June 16, 1998; U.S. Patent 5,753,235, Haanes et al., issued May 19, 1998; and U.S. Patent 5,804,197, Haanes et al., issued September 8, 1998, all of which are incorporated by reference herein in their entireties.

When administered to an animal, a recombinant virus therapy or vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of protecting the animal from a disease. For example, a recombinant virus vaccine comprising a feline IL-18 nucleic acid molecule of the present invention can be administered according to a protocol that results in the animal producing a sufficient immune response to protect itself from a disease mediated by IL-18. In another embodiment of the present invention a feline IL-18 nucleic acid molecule can be used as therapy to treat a disease. A recombinant virus vaccine comprising a feline IL-18 nucleic acid molecule can be administered to an animal with clinical signs of disease according to a protocol that results in reduction and/or termination of clinical signs of disease. A preferred single dose of a recombinant virus therapy or vaccine of the present invention is from about 1×10^4 to about 1×10^8 virus plaque forming units (pfu) per kilogram body weight of the

animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal, topical and oral administration routes being preferred.

A recombinant cell therapy or vaccine of the present invention includes recombinant cells of the present invention that express at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast, (including *Saccharomyces cerevisiae and Pichia pastoris*), BHK, CV-1, myoblast G8, COS, e.g.,COS-7, Vero, MDCK and CRFK recombinant cells. Recombinant cell therapy or vaccines of the present invention can be administered in a variety of ways but have the advantage that they can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. Recombinant cell vaccines can comprise whole cells, cells stripped of cell walls or cell lysates.

In one embodiment of the present invention, a method to regulate an immune response in an animal by administering the therapeutic compound to an animal preferably a canine or feline, wherein the composition comprises a component selected from the group consisting of an excipient, an adjuvant and a carrier.

Proteins of the present invention can be used to develop regulatory compounds including inhibitors and activators that, when administered to an animal in an effective manner, are capable of protecting that animal from disease mediated by IL-18, caspase-1 or IL-12. Preferred regulatory compounds derived from the present invention include inhibitors and activators. In accordance with the present invention, the ability of a

regulatory compound, including an inhibitor or activator, of the present invention to protect a felid or canid from disease mediated by IL-18, caspase-1 or IL-12 refers to the ability of that compound to, for example, treat, ameliorate or prevent a disease mediated by IL-18, caspase-1 or IL-12 in that animal.

An IL-18, caspase-1 or IL-12 single chain inhibitor of the present invention is identified by its ability to bind to, modify, or otherwise interact with, an IL-18, caspase-1 or IL-12 single chain protein of the present invention, thereby inhibiting the activity of the protein. Suitable inhibitors of activity are compounds that inhibit the activity of the proteins of the present invention in at least one of a variety of ways: (1) by binding to or otherwise interacting with or otherwise modifying the protein binding, (2) by interacting with other regions of the protein to inhibit activity, for example, by allosteric interaction, and (3) by binding to or otherwise interacting with or otherwise modifying a protein receptor binding site such that the protein is less likely to bind to the protein receptor binding site. Inhibitors of IL-18, caspase-1 and IL-12 single chain proteins are preferably relatively small compounds.

An embodiment of the present invention includes use of one of the following methods to identify a compound capable of regulating an immune response in an animal: (a) contacting an isolated feline IL-18 protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has T cell stimulating activity; and determining if the putative inhibitory compound inhibits the activity; (b) contacting an isolated feline caspase-1 protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein cleaves precursor IL-18 resulting in a biologically active mature IL-18; and determining if the putative

inhibitory compound inhibits the activity; and (c) contacting an isolated IL-12 single chain protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has T cell proliferation stimulating activity; and determining if the putative inhibitory compound inhibits the activity.

A variety of methods are known to one skilled in the art to detect binding of an IL-18, caspase-1 or IL-12 protein to its binding partner (e.g., an antibody or receptor, as appropriate). Such methods can be used to detect IL-12, casp-1, or IL-18, or Abs or other binding partners thereof in a biological sample or to produce inhibitors of such interactions. Such methods include, but are not limited to an assay in which, for example, IL-18 and an IL-18 binding partner can interact and/or bind to each other, using, for example, the yeast two-hybrid system, see for example, Luban, et al. 1995, *Curr. Opin. Biotechnol.*, 6, 59-64; and identifying those proteins that specifically bind to the IL-18 protein binding domain. Additional methods to identify protein-protein interactions include Biacore® screening, confocal immunofluorescent microscopy, UV cross-linking, and immunoprecipitations. An example of a protein binding domain is an IL-18-binding domain, and a protein that would bind to an IL-18-binding domain would be IL-18. Additional teachings of general characteristics of reagents for use in the detection of binding between two moieties (e.g., between IL-18 and its receptor) as well as methods to produce and use such reagents are disclosed, for example, in United States Patent No. 5,958,880, issued September 28, 1999, by Frank et al.; and PCT International Publication No. WO 99/54349, published October 28, 1999, by McCall et al.; each of these references is incorporated by reference herein in its entirety; furthermore, the disclosed reagents and methods are incorporated by reference herein in their entireties. It

is to be noted that although the reagents and methods disclosed in each of the citations do not relate to the proteins, nucleic acid molecules, antibodies and inhibitors of the present invention per se, the disclosed reagents and methods are applicable by those skilled in the art to reagents, kits and detection methods of the present invention. Furthermore, proteins of the present invention can be used to develop regulatory compounds including inhibitors and activators that, when administered to a canid or felid in an effective manner are capable of protecting and treating that felid or canid from disease mediated by IL-18, caspase-1 or IL-12.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention. The following examples include a number of recombinant DNA and protein chemistry techniques known to those skilled in the art; see, for example, Sambrook et al., *ibid.*

Example 1

Identification of the nucleic acid molecules of the feline IL-18 is unexpected because initial attempts to isolate feline IL-18 nucleic acid molecules using standard cDNA screening techniques were unsuccessful.

This example describes the isolation, sequencing and expression of nucleic acid molecules encoding feline IL-18 proteins of the present invention.

A. Feline IL-18 nucleic acid molecules were isolated as follows: A cDNA mitogen library was prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours as previously described in Example 2 of PCT Publication No. WO 99/61618, entitled "Canine and Feline Immunoregulatory Proteins, Nucleic Acid Molecules, and Uses Thereof," inventors Gek-Kee Sim, Shumin Yang, Matthew Dreitz,

and Ramani Wonderling, filed May 28, 1999, which is incorporated by reference herein in its entirety. An aliquot of this library was used as a template to isolate a feline IL-18 nucleic acid molecule by polymerase chain reaction (PCR). PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc, Foster City, CA). Two overlapping nucleic acid molecules encoding partial length feline IL-18 proteins were obtained by using IL-18 specific primers in combination with cDNA library vector specific primers. All primers came from Life Technologies, Gaithersburg, MD. The sequence of the vector forward primer (T3 primer) was 5' GCCAAGCTCG AAATTAACCC TCACTAAAGG 3' (SEQ ID NO:72), and that of the vector reverse primer (T7 primer) was 5 CGACGGCCAG TGAATTGTAA TACGACTC 3' (SEQ ID NO:73). The sequence of the IL-18-specific forward primer (IL-18 Forward 85) was 5'AGTGATGAAG GCCTGGAATC AGATTACTTT G 3' (SEQ ID NO:74) and the sequence of the IL-18-specific reverse primer (IL-18 Reverse 435) was 5' ATGGCCTGGA ACACTTCTCT GAAAGAATAT GA 3' (SEQ ID NO:75). The first PCR amplification was done using T3 primer and IL-18 Reverse 435 primer and the second PCR amplification was done using IL-18 Forward 85 primer and T7 primer. The PCR profile for both reactions were as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 30 seconds, then 59°C for 30 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. The PCR products from both reactions were cloned into the TA-Cloning vector (available from Invitrogen, San Diego, CA) and the nucleic acid molecules were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator

Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). The PCR product from the first PCR amplification was sequenced and found to contain 514 nucleotides and was denoted herein as nFeIL-18_{514} (5'-end partial clone) with a coding strand of SEQ ID NO:1, and a complementary strand of SEQ ID NO:3. The PCR product from the second PCR amplification was sequenced and found to contain 502 nucleotides and was denoted herein as nFeIL-18_{502} (3'-end partial clone) with a coding strand of SEQ ID NO:4, and a complementary strand of SEQ ID NO:6. These two nucleic acid molecules shared more than 280 base pairs (bp) and together provided the sequence for the complete feline IL-18 open reading frame. Translation of SEQ ID NO:1 suggests that nucleic acid molecule nFeIL-18-N_{514} encodes an N-terminal portion of PFeIL-18-N protein, of about 133 amino acids, denoted herein as PFe IL-18-N_{133}, the amino acid sequence of which is presented in SEQ ID NO:2, assuming an open reading frame having an initiation codon spanning from nucleotide 114 through nucleotide 116 of SEQ ID NO:1 and a stop codon spanning from nucleotide 510 through nucleotide 512 of SEQ ID NO:1. Translation of SEQ ID NO:4 suggests that nucleic acid molecule nFeIL-18-C_{502} encodes an C-terminal portion of PFeIL-18-C protein, of about 154 amino acids, denoted herein as PFe IL-18-C_{154}, the amino acid sequence of which is presented in SEQ ID NO:5, assuming an open reading frame having an initiation codon spanning from nucleotide 3 through nucleotide 5 of SEQ ID NO:4 and a stop codon spanning from nucleotide 462 through nucleotide 464 of SEQ ID NO:4.

Based on the sequence data obtained from these two nucleic acid molecules two new primers were made to isolate a cDNA encoding full-length feline IL-18. The IL-18 Full Forward primer sequence was 5' AACTATTGAG CACAGGGATA

AAGATGACTG 3' (SEQ ID NO:76) and IL-18 Full Reverse primer sequence was 5' AATATCTAAT TCTTGTTTTG AACAGTGAAC ATT 3' (SEQ ID NO:77). The PCR amplification was performed using these two primers and Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.) and an aliquot of the cDNA library prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours. The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 30 seconds, then 53°C for 30 seconds, then 72°C for 90 seconds; followed by a final extension at 72°C for 7 minutes. The PCR product was cloned into the TA-Cloning vector (available from Invitrogen, San Diego, CA) and the nucleic acid molecule insert was sequenced using an ABI PRISM™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using PRISM™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). This PCR product the FeIL-18 containing region of which is denoted nFeIL-18_{607} was found to encode a full-length FeIL-18 protein. The nucleotide sequence of the coding strand of nFeIL-18_{607} is represented herein as SEQ ID NO:7, and its complement is denoted by SEQ ID NO:10. Translation of the open reading frame in SEQ ID NO:7, denoted herein as nFeIL-18_{576}, the coding strand of which is denoted SEQ ID NO: 9, and the complementary strand denoted SEQ ID NO:41 suggests that feline IL-18 encodes a protein containing 192 amino acids, referred to herein as PFeIL-18_{192}, with a SEQ ID NO:8. The nucleic acid sequence encoding feline IL-18 protein assumes an open reading frame in which the first codon spans from nucleotide 24 through 26 of SEQ ID NO:7, and the last codon spans from nucleotide 597 through nucleotide 599 of SEQ ID NO:7. The

encoded protein has a predicted molecular weight of about 21.3 kiloDaltons (kDa) for the precursor protein. The IL-18 precursor protein does not contain a signal sequence; in order for IL-18 to be biologically active the precursor is cleaved by caspase-1. The putative caspase-1 cleavage site is between amino acid positions 35 and 36 of the feline IL-18 precursor protein. Nucleic acid molecule nFeIL-18_{471}, which encodes the mature protein contains a coding strand with SEQ ID NO:11, and a complementary strand with SEQ ID NO:13. The amino acid sequence of the mature protein, denoted herein as PFeIL-18_{157} is SEQ ID NO:12 and the mature protein has a predicted molecular weight of about 17.4 kDa. Sequence analysis was performed using DNAsis™ , available from Hitachi Software, San Bruno, CA using the alignment settings of: gap penalty set at 5, k-tuple set at 3, number of top diagonals set at 5, window size set at 5, fixed gap penalty set at 10 and floating gap penalty set at 10.

B. In an attempt to express a mature feline IL-18 protein in a mammalian cell line, the region encoding only the mature IL-18 protein (SEQ ID NO:11) was isolated from the feline cDNA library described in Example 1A using the following primers: IL-18 MatNgo Forward primer which has the sequence 5' TATGCCGGCT ACTTTGGCAA GCTTGAACAT AAACTC 3' (SEQ ID NO:78) and IL-18 MatXho Reverse primer which has the sequence 5' GGCCTCGAGC TAATTCTTGT TTTGAACAGT GAACATT 3' (SEQ ID NO:79). The PCR amplification was performed using these two primers and Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.) and an aliquot of the cDNA library prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours. The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for

30 seconds, then 53°C for 30 seconds, then 72°C for 90 seconds; followed by a final extension at 72°C for 7 minutes. The PCR products were digested with Ngo MI and Xho I restriction enzymes (available from New England Biolabs, Beverly, MA) and ligated downstream of nucleotides encoding a tissue plasminogen activator (tPA) signal sequence contained in the CMV-IntronA-tPA vector (available from Invitrogen). The construct was sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). This construct encoded the mature feline IL-18 protein with the tPA signal sequence. When Chinese hamster ovary (CHO) cells (available from ATCC, Rockville, MD) were transiently transfected with this construct, using techniques known to those skilled in the art and cell pellets and supernatants were harvested after 48 hrs. Western analysis was performed on the cell pellets and supernatant samples using a polyclonal antibody against human IL-18 (available from Biosource International, Camarillo, CA). A faint band of the expected size (about 17.4 kDa) was detected in the cell pellet and not in the supernatant, indicating that IL-18 is produced by this construct but it is not exported out of the cell at detectable levels. While not being bound by theory, it is believed that caspase-1 plays a key role in the processing of native IL-18 precursor in cells where IL-18 is produced, co-expression of full-length feline IL-18 along with the feline caspase-1 may be necessary for the proper processing of the IL-18 precursor and enhanced secretion of the processed IL-18 mature polypeptide.

Example 2

This example describes the isolation and sequencing of nucleic acid molecules encoding feline caspase-1 proteins of the present invention.

Feline caspase-1 nucleic acid molecules were isolated as follows: A cDNA mitogen library was prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours as described in Example 1. An aliquot of this library was used as a template to isolate a feline caspase-1 by polymerase chain reaction (PCR). PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.). The forward and reverse primers were designed based on human caspase-1 sequences. The forward primer (Casp-1For) had a sequence of 5' ATGGCCGACA AGGTCCTGAA GGAGAAGA 3' (SEQ ID NO:80) and the reverse primer (Casp-1 Rev) had a sequence of 5' TTAATGTCCT GGGAAGAGGT AGAAACATCT TGT 3' (SEQ ID NO:81). The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 45 seconds, then 53°C for 45 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. The PCR product was cloned into the TA-Cloning vector (available from Invitrogen, San Diego, CA) and sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). The PCR product was found to contain the complete full-length feline caspase-1 except for the primer region which was based on the human caspase-1 sequence. The nucleotide sequence of the coding strand of this PCR product is represented herein as nFeCasp-1_{1233}

with a SEQ ID NO: 14, and its complement is denoted by SEQ ID NO:16. Translation of SEQ ID NO:14 suggests that nucleic acid molecule nFeCasp-1_{1233} encodes a full-length nFeCasp-1_{1233} protein, of about 410 amino acids, denoted herein as PFeCasp-1_{410}, the amino acid sequence of which is presented in SEQ ID NO:15, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:14 and a stop codon spanning from nucleotide 408 through nucleotide 410 of SEQ ID NO:14.

Additional primers were made based on the feline caspase-1 sequence of nFeCasp-1_{1233} in order to obtain two nucleic acid molecules spanning the 5' and 3' end of the feline caspase-1 open reading frame. Two feline caspase-1 nucleic acid molecules were generated using feline caspase-1 specific primers in combination with cDNA library vector specific primers. The sequence of the vector forward primer (T3 primer) was 5' GCCAAGCTCG AAATTAACCC TCACTAAAGG 3' (SEQ ID NO:72), and that of the vector reverse primer (T7 primer) was 5' CGACGGCCAG TGAATTGTAA TACGACTC 3' (SEQ ID NO:73). The sequence of the feline caspase-1-specific forward primer (Casp 271 Forward) was 5' TCAAGCCCAC AATCTGGAAA TTCTCA 3'(SEQ ID NO:82) and the sequence of the feline caspase-1-specific reverse primer (Casp 895 Reverse) was 5' CTGGAGAGTC ACTGATCAAC AGTTCC 3'(SEQ ID NO:83). The first PCR amplification was done using T3 primer and Casp 895 Reverse primer and the second PCR amplification was done using Casp 271 Forward primer and T7 primer. The PCR profile for both reactions was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 45 seconds, then 52°C for 45 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. The PCR

products from both reactions that were greater than or equal to 1 kb were gel purified and cloned into the TA-Cloning vector (available from Invitrogen) and the nucleic acid molecules were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). The nucleic acid molecules obtained from these two PCR products represented two nucleic acid molecules of feline caspase-1. The region of the first PCR amplification was sequenced and found to contain 527 nucleotides denoted herein as nFeCasp-1-N_{527} (5'-end partial clone) with a coding strand of SEQ ID NO: 17, and a complementary strand of SEQ ID NO: 19. The region of the second PCR amplification was sequenced and found to contain 500 nucleotides denoted here as nFeCasp-1-C_{500} (3'-end partial clone) with a coding strand of SEQ ID NO: 20, and a complementary strand of SEQ ID NO: 22. Translation of SEQ ID NO:17 suggests that nucleic acid molecule nFeCasp-1-N_{526} encodes an N-terminal portion of PFeCasp-1-N protein, of about 169 amino acids, denoted herein as PFeCasp-1-N_{169}, the amino acid sequence of which is presented in SEQ ID NO:18, assuming an open reading frame having an initiation codon spanning from nucleotide 18 through nucleotide 20 of SEQ ID NO:17 and a stop codon spanning from nucleotide 522 through nucleotide 524 of SEQ ID NO:17. Translation of SEQ ID NO:20 suggests that nucleic acid molecule nFeCasp-1-C_{500} encodes an C-terminal portion of PFeCasp-1-C protein, of about 120 amino acids, denoted herein as PFeCasp-1-C_{120} , the amino acid sequence of which is presented in SEQ ID NO:21, assuming an open reading frame having an initiation codon spanning from

nucleotide 3 through nucleotide 5 of SEQ ID NO:20 and a stop codon spanning from nucleotide 360 through nucleotide 362 of SEQ ID NO:20

Based on the sequence data obtained from nucleic acid molecules nFeCasp-1-N_{527} and nFeCasp-1-C_{500}, two new primers were made to isolate a cDNA encoding full-length feline caspase-1. The feline caspase-1 full-length forward primer (CaspBamKozFor) sequence was 5' ACAAGGATCC ACCATGGCCG ACAAGGATCT GAAGGG 3' (SEQ ID NO:84) and feline caspase-1 full-length reverse primer (CaspXbaRev) sequence was 5' CGCCTCTAGA CCTCAATTGC CAGGGAAGAG ATAGAAGTA 3' (SEQ ID NO:85). The PCR amplification was performed using these two primers and Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.) and an aliquot of the cDNA mitogen library prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours. The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 45 seconds, then 52°C for 45 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. The PCR product was cloned into the TA-Cloning vector (available from Invitrogen) and the nucleic acid molecule inserts were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from PE Applied Biosystems Inc.). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). This PCR product the FeCaspase-1 containing region of which is denoted nFeCasp-1_{1230} was found to encode the a full-length feline caspase-1 protein. The nucleotide sequence of the coding strand of nFeCasp-1_{1230} is represented herein as SEQ ID NO 23, and its complement is denoted by SEQ ID NO:25. Translation of the open reading frame in SEQ

ID NO:23, denoted here as nFeCasp-1_{1230}, the coding strand of which is denoted SEQ ID NO:25 suggests that feline caspase-1 encodes a protein containing 410 amino acids, referred to herein as PFeCasp-1_{410}, with a SEQ ID NO:24. The nucleic acid sequence encoding the protein assumes an open reading frame in which the first codon spans from nucleotide 1 through 3 of SEQ ID NO:23, and the last codon spans from nucleotide 1228 nucleotide 1230 of SEQ ID NO:23. The encoded protein has a predicted molecular weight of about 45.5 kDa. The feline caspase-1 protein is 9 amino acids longer than mouse and rat caspase-1 proteins, 6 amino acids longer than dog and human caspase-1 proteins, and 5 amino acids longer than horse caspase-1 proteins. Sequence analysis was performed using DNAsis™ , available from Hitachi Software, San Bruno, CA using the alignment settings of: gap penalty set at 5, k-tuple set at 3, number of top diagonals set at 5, window size set at 5, fixed gap penalty set at 10 and floating gap penalty set at 10.

Example 3

This example describes the isolation and sequencing of nucleic acid molecules encoding feline IL-12 single chain proteins of the present invention.

A. A pBluescript-Linker plasmid was constructed as follows: Two complementary oligonucleotides, 60 nucleotides in length were synthesized. The oligonucleotides were allowed to hybridize to each other in solution producing a double stranded DNA fragment that would serve as a linker between the cDNAs encoding the p40 and p35 subunits of feline IL-12. The sequence of the sense linker was 5' CTGCAGTGGT GGCGGTGGCG GCGGATCTAG AAACTTGCCA ACCCCTACTC CATCCCCGGG 3' (SEQ ID NO:83) and the sequence of the antisense linker was 5' CCCGGGGATG GAGTAGGGGT TGGCAAGTTT CTAGATCCGC CGCCACCGCC

ACCACTGCAG 3' (SEQ ID NO:84). Equimolar amounts of sense linker and antisense linker were mixed and heated to 95°C for 10 minutes in a heat block. The heat block containing the samples was removed from the heat source and allowed to cool to room temperature slowly, over a period of 4 hours. Then the hybridized oligonucleotides were digested with *Pst*I and *Sma*I restriction enzymes (available from New England Biolabs, Beverly, MA) and ligated into pBluescript SK+ vector (available from Stratagene, La Jolla, CA) digested with the same restriction enzymes to produce pBluescript-Linker plasmid. The presence of the linker in the ligated pBluescript-Linker plasmid was confirmed by sequencing conducted as described in Example 1. The pBluescript-Linker plasmid contained DNA coding for the following elements: (1) the last two C-terminal amino acid residues of the p40 subunit (i.e. C,S); (2) the seven amino acid residues of the linker (i.e. GGGGGGS); and (3) the first ten N-terminal amino acid residues of the mature p35 subunit mature protein (i.e. RNLPTPTPSP).

B. Feline IL-12 p40 nucleic acid molecule subunit was isolated as follows: A cDNA mitogen library was prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours as previously described in Example 1. An aliquot of this library was used as a template to isolate a feline IL-12 p40 nucleic acid molecule subunit by polymerase chain reaction (PCR). The PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.). The sequence of the forward primer was 5' ATGCATCCTC AGCAGTTGGT CATCGCCT 3' (SEQ ID NO:85), and that of the reverse primer was 5' TGCAGGACAC GGATGCCCAG TTGCT 3' (SEQ ID NO:86). The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the following: 94°C for 45 seconds, then 50°C for

45 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. PCR products were cloned into the TA-Cloning vector (available from Invitrogen) and the nucleic acid molecule inserts were sequenced as described in Example 1. One of the sequenced PCR products contained 985 nucleotides and was denoted herein as nFeIL-12 p40-N_{985} with a coding strand of SEQ ID NO:55, and a complementary strand of SEQ ID NO:57. Translation of SEQ ID NO:55 suggests that nucleic acid molecule nFeIL-12p40-N_{985} encodes an N-terminal portion of PFeIL-12p40-N protein, of about 328 amino acids, denoted herein as PFe IL-12p40-N_{328}, the amino acid sequence of which is presented in SEQ ID NO:56, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:55 and a stop codon spanning from nucleotide 982 through nucleotide 984 of SEQ ID NO:55.

This nucleic acid molecule was used as a template for a subsequent PCR reaction to obtain a full-length nucleic acid molecule. The PCR amplification was performed using Amplitaq DNA polymerase ™ (PE Applied Biosystems Inc, Foster City, CA). The sequence of the forward primer was 5' ACAGGTACCA TGCATCCTCA GCAGTTGGTC ATCGCCT 3' (SEQ ID NO:87), and that of the reverse primer was 5' CTAACTGCAG GACACGGATG CCCAG 3' (SEQ ID NO:88). The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 35 cycles of the following: 94°C for 30 seconds, then 50°C for 30 seconds, then 72°C for 90 seconds; followed by a final extension at 72°C for 7 minutes. This PCR product, the Fe IL-12p40 single chain subunit containing region of which is denoted nFeIL-12 $p40_{987}$ was found to encode a full-length feline IL-12 p40 single chain subunit protein. The nucleotide sequence of the coding strand of nFeIL-12 $p40_{987}$ is represented herein as SEQ ID NO:29,

and its complementary strand is denoted by SEQ ID NO:31. Translation of SEQ ID NO:29 suggests that nucleic acid molecule nFeIL-12p40$_{987}$ encodes a full-length PFeIL-12p40 protein of about 329 amino acids, denoted herein as PFe IL-12p40$_{329}$, the amino acid sequence of which is presented in SEQ ID NO:30, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:29 and a stop codon spanning from nucleotide 985 through nucleotide 987 of SEQ ID NO:29. This PCR product was digested with *Kpn* I and *Pst* I restriction enzymes (available from New England Biolabs) and cloned into the pBluescript-Linker plasmid described in Example 3A. The resultant recombinant molecule is referred to as fep40-linker plasmid. There is a putative cleavage site on SEQ ID NO:30, yielding the coding region for a mature (i.e. lacking a signal or leader sequence) nFeIL-12p40$_{921}$, denoted herein as SEQ ID NO:26, with the complement denoted SEQ ID NO:28. Translation of SEQ ID NO:26 yields a mature IL-12 p40 protein denoted PFeIL-12p40$_{307}$, also denoted herein as SEQ ID NO:27.

C. A Feline IL-12 p35 nucleic acid molecule subunit was isolated as follows: A cDNA mitogen library was prepared from cat peripheral blood lymphocytes stimulated with ConA for 4 hours as previously described in Example 1. An aliquot of this library was used as a template to isolate feline IL-12 p35 subunit by polymerase chain reaction (PCR). The PCR amplification was performed using Amplitaq DNA polymerase ™ (PE Applied Biosystems Inc, Foster City, CA). The sequence of the forward primer was 5' ATGTGCCCGC CGCGTGGCC 3' (SEQ ID NO:89), and that of the reverse primer was 5' CTAGGAAGCA TTCAGATAGC TCATCAT 3' (SEQ ID NO:90). The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 43 cycles of the

following: 94°C for 45 seconds, then 50°C for 45 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. PCR products were cloned into the TA-Cloning vector (available from Invitrogen) and the nucleic acid molecules were sequenced as described in Example 1. One of the sequenced PCR products contained 666 nucleotides and was denoted herein as nFeIL-12-p35$_{666}$ with a coding strand of SEQ ID NO:32, and a complementary strand of SEQ ID NO:34. Translation of SEQ ID NO:32 suggests that nucleic acid molecule nFeIL-12p35$_{666}$ encodes a full-length PFeIL-12p35 protein of about 222 amino acids, denoted herein as PFe IL-12p35$_{222}$, the amino acid sequence of which is presented in SEQ ID NO:33, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:32 and a stop codon spanning from nucleotide 664 through nucleotide 666 of SEQ ID NO:32. There is a putative cleavage site on SEQ ID NO:33, yielding the coding region for a mature (i.e. lacking a signal or leader sequence) nFeIL-12p35$_{591}$, denoted herein as SEQ ID NO:35, with the complement denoted SEQ ID NO:37. Translation of SEQ ID NO:26 yields a mature IL-12 p35 protein denoted PFeIL-12p35$_{197}$, also denoted herein as SEQ ID NO:36. SEQ ID NO:26 was digested with Sma I and Not I restriction enzymes (available from New England Biolabs) and cloned into the fep40-linker plasmid described in Example 3B digested with the same enzymes. The resultant recombinant molecule is referred to as fep40-linker-p35mature plasmid.

D. The fep40-linker-p35mature plasmid contained a nucleic acid molecule encoding a feline IL-12 single chain protein of the present invention inserted into the Kpn I and Not I sites of the pBluescript backbone. Nucleic acid molecule nFeIL-12$_{1599}$ was sequenced as described in Example 1. The nucleotide sequence of the coding strand of

nFeIL-12_{1599} is represented herein as SEQ ID NO:38, and that of the complementary strand is SEQ ID NO:40. Translation of the open reading frame in SEQ ID NO:38, suggests that nFeIL-12_{1599} encodes a protein containing 533 amino acids, referred to herein as pFeIL-12_{533}, with an amino acid sequence denoted by SEQ ID NO:39. The nucleic acid sequence encoding the protein assumes an open reading frame in which the first codon spans from nucleotide 1 through 3 of SEQ ID NO:38 and the last codon spans from nucleotide 1597 nucleotide 1599 of SEQ ID NO:38. The encoded protein has a predicted molecular weight of about 59.2 kDa. The putative signal peptide cleavage site is between amino acid positions 22 and 23 of the p40 subunit. Nucleic acid molecule nFeIL-12_{1533} which encodes the mature protein contains a coding strand with SEQ ID NO:43, and a complementary strand with SEQ ID NO:45. The amino acid sequence of the mature protein, denoted herein as PFeIL-12_{511} is SEQ ID NO:44 and the mature protein has a predicted molecular weight of about 56.8 kDa.

Chinese hamster ovary (CHO) cells (available from ATCC, Rockville, MD) were transiently transfected with fep40-linker-p35mature plasmid (containing SEQ ID NO:38) using techniques known to those skilled in the art, cell pellets and supernatants were harvested after 48 hrs. Western analysis was performed on the cell pellets and supernatant samples using a polyclonal antibody against human IL-12 (available from Biosource International, Camarillo, CA). A faint band of the expected size (about 59.2 kDa) was detected in the cell pellet and in the supernatant, indicating that IL-12 is produced by this construct at detectable levels.

Example 4

This example describes the isolation and sequencing of nucleic acid molecules encoding canine IL-12 single chain proteins of the present invention.

A. A canine IL-12 p35 nucleic acid molecule subunit was isolated as follows: A cDNA mitogen library was prepared from canine peripheral blood lymphocytes (PBLs) stimulated with ConA for 4 hours as described in Example 1. Recombinant phage containing DNA encoding the p35 subunit were identified by nucleic acid hybridization using a P^{32} radiolabeled probe. The p35 probe (nCaIL-12p35TA) was generated by PCR of total RNA, prepared from ConA-stimulated PBLs in the following manner. The sequence of the forward primer was 5' CCATCCTGGT CCTGCTAAG C 3' (SEQ ID NO:93) and the sequence of the reverse primer was 5' CCATCTGGTA CATCTTCAAG TC 3' (SEQ ID NO:94). PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.) using the following profile: 95°C for 2 minutes; then 30 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; and a final extension at 72°C for 10 minutes. The amplified DNA fragment was purified with Qiagen gel purification kit, available from Qiagen, La Jolla, CA) and PCR products were cloned into the TA cloning vector (available from Invitrogen Corporation, Carlsbad, CA), and the resulting clones were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from Perkin-Elmer Applied Biosystems Inc., Foster City, CA). DNA sequencing reactions were performed using Prism™ dRhodamine Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). Phage DNA was permanently cross-linked to the nitrocellulose sheets using a Stratalinker(R) UV crosslinker (available from Stratagene).

The plaque lifts were pre-hybridized in a solution of 6 X SSC (20 X SSC is 3.0 M NaCl and 0.3 M sodium citrate), 5 X Denhardt's solution (50 X SSC is 0.01 grams/milliliter Ficoll, type 400; 0.01g/ml polyvinylpyrrolidone; and 0.01g/ml bovine serum albumin, fraction V, all available from Sigma, St. Louis, MO), 0.5% sodium dodecyl sulfate (SDS), and 100 micrograms/ml denatured salmon sperm for 2 hours at 68°C. Denatured, radiolabeled probe was added to the pre-hybridization solution at a concentration of $1x10^6$ cpm/ml and the hybridization continued for 18-24 hours at 68°C. Nonspecifically bound and unbound probe was removed by washing two times in 2 X SSC with 0.1% SDS, 30 minutes each at 68°C and one time in 1 X SSC with 0.1% SDS, 60 minutes at 68°C. The hybridized plaque lifts were exposed to Kodak x-ray film for approximately 18 hours. Positive phage were plaque purified three times using the following hybridization protocol: phage plaques grown in solid top agar were lifted onto pure nitrocellulose sheets (available from Schleicher & Schuell, Keene, NH) then denatured and neutralized by soaking the sheets in 0.5 N NaOH /1.5 M NaCl, followed by 0.5 M Tris-HCl pH7.4 /1.5 M NaCl. pBluescript plasmid, containing a cDNA encoding the full-length canine IL-12 p35 subunit, was excised from plaque purified phage using the ExAssist™ helper phage (available from Stratagene) following the manufacturers' instructions. The nucleotide sequence of that cDNA, denoted herein as nCa IL-12 $p35_{1455}$ was verified by sequencing as described in Example 1. The nucleic acid sequence of the coding strand of nCaIL-$12p35_{1455}$ represented as SEQ ID NO: 104, and its complementary strand is SEQ ID NO:106. Translation of SEQ ID NO:104 suggests that nucleic acid molecule nCaIL-$12p35_{1455}$ encodes an N-terminal portion of PCaIL-12p35 protein, of about 222 amino acids, denoted herein as PCa IL-$12p35_{222}$, the amino acid sequence of which is presented

in SEQ ID NO:105, assuming an open reading frame having an initiation codon spanning from nucleotide 232 through nucleotide 234 of SEQ ID NO:104 and a stop codon spanning from nucleotide 895 through nucleotide 897 of SEQ ID NO:104.

Nucleic acid molecule nCaIL-12p35$_{1455}$ was used as the template in PCR to obtain the coding region of the full-length form of canine IL-12 p35 subunit. The sequence of the forward primer was 5' AAAAAACCCG GGTATGTTCC AATGTTTCAA CCACTCCC 3' (SEQ ID NO:95) and the sequence of the reverse primer was 5' GCGGCCGCTC GAGTTAGGAA GAGTTCAAGT AGGACATCAT TCTATTGATG G 3' (SEQ ID NO:96). PCR was performed using *Pfu* DNA polymerase (available from Stratagene) as follows: 95°C for 45 seconds; then 25 cycles of 95°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute; followed by a final extension at 72°C for 10 minutes. The PCR product contains the nucleic acid sequence of canine IL-12 p35 subunit which encodes a full-length canine IL-12 p35 subunit protein. The nucleotide sequence of the coding strand of nCaIL-12p35$_{666}$ is represented herein as SEQ ID NO:46 and its complementary strand is denoted SEQ ID NO:48. Translation of SEQ ID NO:46 suggests that nucleic acid molecule nCaIL-12p35$_{666}$ encodes a mature PCaIL-12p35 single chain protein of about 222 amino acids, denoted herein as PCaIL-12p35$_{222}$, the amino acid sequence of which is presented in SEQ ID NO:47, assuming an open reading frame having an initiation codon spanning from nucleotide 1 through nucleotide 3 of SEQ ID NO:46 and a stop codon spanning from nucleotide 589 through nucleotide 591 of SEQ ID NO:46. The coding sequence for the mature polypeptide is encoded by SEQ ID NO:46, the coding region for a mature (i.e. lacking a signal or leader sequence) nCaIL-12p35$_{591}$, denoted herein as SEQ ID NO:49, with the complement denoted SEQ ID

NO:51. Translation of SEQ ID NO:49 yields a mature IL-12 p35 protein denoted PCaIL-12p35$_{197}$, also denoted herein as SEQ ID NO:50. nCaIL-12p35$_{591}$ was digested with *Sma*I and *Xho*I restriction endonucleases (available from New England Biolabs) and ligated into the pBluescript-Linker plasmid described in Example 3A digested with the same enzymes. The resultant recombinant molecule is referred to as calinker-p35mature plasmid.

B. Canine IL-12 p40 nucleic acid molecule subunit was isolated as follows: A cDNA mitogen library was prepared from canine peripheral blood lymphocytes stimulated with ConA for 4 hours as described in Example 1. Recombinant phage containing DNA encoding the p40 subunit were identified by nucleic acid hybridization using a P^{32} radiolabeled probe. The p40 probe (nCaIL-12p40TA) was generated by PCR of total RNA, prepared from ConA stimulated PBLs in the following manner. The sequence of the forward primer was 5' CTTAAAGGAA CAGAAAGAAT CC 3' (SEQ ID NO:97) and the sequence of the reverse primer was 5' GGTATTCCCA GCTGACCTC 3' (SEQ ID NO:98). PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.) using the following profile: 95°C for 2 minutes; then 30 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; and a final extension at 72°C for 10 minutes. The amplified DNA fragment was purified with Qiagen gel purification kit, available from Qiagen, La Jolla, CA) and PCR products were cloned into the TA cloning vector (available from Invitrogen Corporation, Carlsbad, CA), and the resulting clones were sequenced using an ABI Prism™ Model 377 Automatic DNA Sequencer (available from Perkin-Elmer Applied Biosystems Inc., Foster City, CA). DNA sequencing reactions were performed using Prism™ dRhodamine

Terminator Cycle Sequencing Ready Reaction kits (available from PE Applied Biosystems Inc.). Phage DNA was permanently cross-linked to the nitrocellulose sheets using a Stratalinker(R) UV crosslinker (available from Stratagene). The plaque lifts were pre-hybridized in a solution of 6 X SSC (20 X SSC is 3.0 M NaCl and 0.3 M sodium citrate), 5 X Denhardt's solution (50 X SSC is 0.01 grams/milliliter Ficoll, type 400; 0.01g/ml polyvinylpyrrolidone; and 0.01g/ml bovine serum albumin, fraction V, all available from Sigma, St. Louis, MO), 0.5% sodium dodecyl sulfate (SDS), and 100 micrograms/ml denatured salmon sperm for 2 hours at 68°C. Denatured, radiolabeled probe was added to the pre-hybridization solution at a concentration of $1x10^6$ cpm/ml and the hybridization continued for 18-24 hours at 68°C. Nonspecifically bound and unbound probe was removed by washing two times in 2 X SSC with 0.1% SDS, 30 minutes each at 68°C and one time in 1 X SSC with 0.1% SDS, 60 minutes at 68°C. The hybridized plaque lifts were exposed to Kodak x-ray film for approximately 18 hours. Positive phage were plaque purified three times using the following hybridization protocol: phage plaques grown in solid top agar were lifted onto pure nitrocellulose sheets (available from Schleicher & Schuell, Keene, NH) then denatured and neutralized by soaking the sheets in 0.5 N NaOH /1.5 M NaCl, followed by 0.5 M Tris-HCl pH7.4 /1.5 M NaCl. pBluescript plasmid, containing a cDNA encoding the full-length canine IL-12 p40 subunit, was excised from plaque purified phage using the ExAssist™ helper phage (available from Stratagene) following the manufacturers' instructions. The nucleotide sequence of that cDNA, denoted herein as nCaIL-$12p40_{2267}$ was verified by sequencing as described in Example 1. The nucleic acid sequence of the coding strand of nCaIL-$12p40_{2267}$ represented as SEQ ID NO:107, and its complementary strand is SEQ ID

NO:109. Translation of SEQ ID NO:107 suggests that nucleic acid molecule nCaIL-12p40$_{2267}$ encodes an PCaIL-12p40 protein, of about 329 amino acids, denoted herein as PCa IL-12p40$_{329}$, the amino acid sequence of which is presented in SEQ ID NO:108, assuming an open reading frame having an initiation codon spanning from nucleotide 154 through nucleotide 156 of SEQ ID NO:107 and a stop codon spanning from nucleotide 1138 through nucleotide 1140 of SEQ ID NO:107. Full length canine IL-12 p40 nucleic acid molecule was isolated as follows: A plasmid containing full-length canine IL-12 p40 nucleic acid molecule subunit (pCaIL-12p40) was used as a template to sub-clone canine IL-12 p40 subunit by polymerase chain reaction (PCR). PCR amplification was performed using Amplitaq DNA polymerase™ (available from PE Applied Biosystems Inc.). The sequence of the forward primer (Dog p40 KpnFor) was 5' CATAGGTACC ATGCACCCTC AGCAGTTGGT CATCTCC 3' (SEQ ID NO:99), and that of the reverse primer (Dog p40 NsiRev) was 5' ATCTAAATGC ATGACACAGA TGCCCAGTC 3' (SEQ ID NO: 100). The PCR profile was as follows: one initial denaturation step at 94°C for 5 minutes; then 35 cycles of the following: 94°C for 30 seconds, then 55°C for 30 seconds, then 72°C for 2 minutes; followed by a final extension at 72°C for 7 minutes. The PCR product contains the nucleic acid sequence of canine IL-12 p40 subunit along with its native signal sequence which encodes a canine full-length IL-12 p40 subunit protein. The nucleotide sequence of the coding strand of nCaIL-12 p40$_{987}$ is represented herein as SEQ ID NO:58 and its complementary strand is denoted SEQ ID NO:60. Translation of the open reading frame in SEQ ID NO:58, suggests that canine IL-12 p40 subunit encodes a protein containing 329 amino acids, referred to herein as PCaIL-12 p40$_{329}$ with an amino acid sequence denoted by SEQ ID NO:59. The resulting

recombinant molecule is referred to as cap40-linker plasmid. The cap40-linker plasmid was digested with Kpn I and Pst I restriction enzymes (available from New England Biolabs) to remove the region encoding canine p40 mature protein. The PCR product containing the full-length canine p40 subunit (nCaIL-12 $p40_{987}$) was digested with *Kpn* I and *Nsi* I restriction enzymes (available from New England Biolabs) and cloned into this digested plasmid. The coding sequence for the mature canine IL-12 p40 polypeptide is encoded by SEQ ID NO:52, the coding region for a mature (i.e. lacking a signal or leader sequence) nCaIL-12$p40_{921}$, with the complement denoted SEQ ID NO:53. Translation of SEQ ID NO:52 yields a mature IL-12 p40 protein denoted PCaIL-12$p35_{307}$, also denoted herein as SEQ ID NO:53. nCaIL-12$p40_{921}$ was digested with *Sma*I and *Xho*I restriction endonucleases (available from New England Biolabs) and ligated into the pBluescript-Linker plasmid described in Example 3A digested with the same enzymes.

The resulting plasmid contains a nucleic acid molecule encoding a canine IL-12 single chain cloned at the *Kpn* I and *Not* I site into the pBluescript backbone. The complete canine IL-12 single chain insert was sequenced as described in Example 1. The nucleotide sequence of the coding strand of nCaIL-12-single $chain_{1599}$ is represented herein as SEQ ID NO:61, and its complement is denoted by SEQ ID NO:63. Translation of the open reading frame in SEQ ID NO:61, denoted here as nCaIL-12_{1599} with a SEQ ID NO:64 suggests that canine IL-12-single chain encodes a protein containing 533 amino acids, referred to herein as PCaIL-12_{533}, with an amino acid sequence denoted by SEQ ID NO:62, assuming an open reading frame in which the first codon spans from nucleotide 1 through 3 of SEQ ID NO:61 and the last codon spans from nucleotide 1597 nucleotide 1599 of SEQ ID NO:61. The encoded protein has a predicted molecular weight of about

59.2 kDa for the precursor protein. and about 56.8 kDa for the mature protein. The putative signal peptide cleavage site is between amino acid positions 22 and 23 of the canine p40 subunit protein. Nucleic acid molecule nCaIL-$12_{1533,}$ which encodes the mature protein contains a coding strand with SEQ ID NO:66, and a complementary strand with SEQ ID NO:68. The amino acid sequence of the mature protein, denoted herein as pCaIL-12_{511}, is SEQ ID NO:67 and the mature protein has a predicted molecular weight of about 56.8 kDa.

Chinese hamster ovary (CHO) cells (available from ATCC, Rockville, MD) were transiently transfected with cap40-linker-p35mature plasmid using techniques known to those skilled in the art and cell pellets and supernatants were harvested after 48 hrs. Western analysis was performed on the cell pellets and supernatant samples using a polyclonal antibody against human IL-12 (available from Biosource International, Camarillo, CA). A faint band of the expected size (about 59.2kDa) was detected in the cell pellet and in the supernatant, indicating that IL-12 is produced by this construct at detectable levels.